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CONTENTS

Studies on control of black rot of crucifers by treating seeds with antibiotics — J. M. Klisiewicz and G. S. Pound 495

Severity and distribution of cotton boll rots as related to temperature — P. M. Halisky, W. C. Schnathorst, and M. A. Shagrun 501

Potential pathogenicity of Pythium isolates from forest nurseries — O. Vaartaja and P. J. Salisbury 505

Physical barriers associated with resistance in Fusarium wilt of bananas — C. H. Beckman, M. E. Mace, S. Halmos, and M. W. McGahan 507

Aphid transmission of barley yellow dwarf virus in Wisconsin — G. B. Orlob, D. C. Arny, and J. T. Medler 515

Rate of reproduction of Meloidogyne spp. as influenced by soil temperature — Ivan J. Thomason and Bert Lear 520

Girdling of grapevine canes by Melanconium fuligineum — P. D. Critopoulos 524

Viscosity of naphthenic and paraffinic spray oils in relation to phytotoxicity and Sigatoka disease control on banana leaves — L. Calpouzos, N. E. Delfel, C. Colberg, and T. Theis 528

An anomalous effect of pentachloronitrobenzene on the incidence of damping-off caused by a Pythium spp. — Ian A. S. Gibson, M. Ledger, and E. Boehm 531

Peroxidase activity in virus-infected sweet potatoes — G. Loebenstein and N. Linsey 533

Race groups of Tilletia caries and Tilletia foetida for varietal-resistance testing — E. L. Kendrick 537

Properties and serology of purified cauliflower mosaic virus — T. P. Pirone, G. S. Pound, and R. J. Shepherd 541

Sowbane mosaic caused by a seed-transmitted virus — C. W. Bennett and A. S. Costa 546

A mutation for resistance to potato virus Y in pepper — A. A. Cook 550

Microorganisms on cucumber seedlings — Curt Leben 553

Tree nutrition and fire blight development — K. G. Parker, N. S. Luepschen, and E. G. Fisher 557

Antihost serum improves plant virus purification — A. Herbert Gold 561

A seed-borne bean virus of wide host range — C. B. Skotland and D. W. Burke 565

Effect of variation in the bacterial spot pathogen of pepper and tomato on control with streptomycin — P. L. Thayer and R. E. Stall 568

Relation of temperature to spore germination, growth, and disease development by Gilbertella persicaria — Joseph M. Ogawa, Edward E. Butler, and Stuart D. Lyda 572

Report and abstracts of the 1960 meeting of the Northeastern Division of The American Phytopathological Society 574

Phytopathological Notes

The possible relation of manganese to stem cankers in red oak — Eldon W. Ross 579

A comparison of wound-tumor and tobacco club-root — M. I. Selsky 581

Deposit rate and spray oil composition in relation to phytotoxicity and Sigatoka disease control of banana leaves — L. Calpouzos, C. Colberg, T. Theis, and N. E. Delfel 582

Injuries on cantaloupe leaves associated with laminal guttation away from marginal hydathodes — S. S. Ivanoff 584

News 586

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STUDIES ON CONTROL OF BLACK ROT OF CRUCIFERS BY TREATING SEEDS WITH ANTIBIOTICS

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SUMMARY

Aqueous solutions of Terramycin, Agri-mycin, streptomycin, Aureomycin, Achromycin, mercuric chloride, and antibiotic combinations were tested in 30-minute seed treatments for control of black rot disease of crucifers in laboratory and greenhouse experiments. Maximum inhibition of *Xanthomonas campestris in vitro* was obtained with the chemical agents at a concentration of approximately 3,000 ppm. Complete disinfection of naturally-infected cabbage and broccoli seed was not obtained with concentrations as high as 3,000 ppm; however, the percentage of seedling infection was reduced to less than 1%. Use of various antibiotic combinations resulted in increased inhibition and seed disinfection. Complete disinfection of infected seed apparently is hindered by lack of absorption into the seed, bacteriostatic action of the tetracyclines, and resistance of *X. campestris* to streptomycin.

In a critical assay of seed infection, a temperature of 28°C, 24-hour photoperiod, moisture chamber exposure of seedlings after emergence, and soaking seed in distilled water for at least 8 hours prior to planting are conditions optimal for disease incidence and development in crucifer seedlings.

Phytotoxicity, manifested as chlorosis and stunting, was generally prevalent on seedlings grown from treated seed and increased in severity with increased concentrations and length of treatment. Monobasic sodium and potassium phosphate in solution were markedly effective in reducing injury caused by the tetracyclines when applied as seed treatments, without hindering antibiotic activity.

Antibiotic sprays applied on cabbage plants at least once prior to inoculation were more effective in reducing disease incidence than 2 or 3 post-inoculation sprays.

INTRODUCTION.—Black rot of crucifers, caused by the bacterium *Xanthomonas campestris* (Pam.) Dowson, occurs throughout the world but is limited or practically absent in regions of low rainfall. The black rot bacteria contaminate the seed by systemic invasion of the seed stalks, invade the seed coat through the vascular system of the funiculus, and are reported to remain viable in naturally infected seed for 3 years (2, 3).

Control of black rot has been a difficult problem, but the use of disease-free seed, seedbed rotation, and seed disinfection by hot water treatment have enhanced efforts to reduce incidence of the disease. Sutton and Bell (7) found a 30-minute treatment of artificially inoculated rutabaga seed with Aureomycin solution at 1,000 ppm effectively controlled the black rot organism in laboratory, greenhouse, and field tests.

This investigation was undertaken with the objective of providing a control for black rot by seed treatment with streptomycin sulfate, Agri-mycin 100, Terramycin hydrochloride, Aureomycin hydrochloride, and Achromycin hydrochloride.

METHODS AND MATERIALS.—Sources of infected seed were seed lots harvested from field-grown cabbage and broccoli seed plants inoculated prior to flowering, and commercial cabbage seed artificially inoculated by soaking in a heavy bacterial suspension for 5 hours.

Antibiotic solutions were prepared by dissolving the antibiotics in distilled water, except in phytotoxicity studies where the solvent was a specific salt solution. Seed samples were placed in small cheesecloth bags and immersed in the antibiotic solution for 30 minutes. The filter paper disc-agar diffusion method, as described by Grove and Randall (5), was used in assays to determine inhibitory levels of the antibiotics on *X. campestris*. Pyrex plates, 8½ x 13 in., were flooded with 225 ml of nutrient-dextrose agar seeded with 10 ml of a broth culture of *X. campestris* with an optical density of 3.5 at 620 mμ. Paper discs dipped in antibiotic solution were allowed to dry and then were placed on the solidified agar. Inhibition zones were measured after 20 hours' incubation at 28°C with fine-point calipers and recorded as averages of 4-disc replicates.

Seed assays similar to the paper disc assay were made to determine antibiotic absorption by treated cabbage seed; however, the agar was seeded with a specific organism sensitive to the antibiotic assayed. Whole seed, seed coats, and halved seed from which the seed coats were removed were plated and zone measurements made after 16-20 hours' incubation at optimum growth temperature. Test organisms used were *Bacillus subtilus* and *B. cereus* var. *mycoides* for streptomycin and Terramycin, respectively; and *Sarcina lutea* for Aureomycin and Achromycin.

In comparative laboratory tests for control of *X. campestris*, naturally infected seed was plated after antibiotic treatment on approximately 10 ml of agar in petri plates and incubated at 28°C for 7-8 days. Untreated seed soaked in distilled water was used as a control. Similar tests were conducted with artificially inoculated seed, except the plates were examined daily during an 18-day period for development of bacterial colonies.

A growth chamber in which temperature and light conditions could be controlled was constructed and

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enclosed with plastic screen. Temperatures of 24°, 28°, and 30°C were thermostatically controlled by use of a strip heater in a series of experiments. A 24-hour photoperiod of approximately 200 ft-c was maintained over the growth chamber. Nutrient levels used were solutions of 0.05H, 0.1H, 0.5H, 1H (Hoagland's basic solution), 2H, and 3H. Cabbage seedlings were grown from naturally infected seed planted in 4-in. varnished clay pots filled with a 2:1 sand-vermiculite mixture. After seedling emergence, 100 ml of nutrient solution were added to each pot and the plants were placed in a moisture chamber for a 12-hour period; seedlings were observed for symptoms over a 14-day period.

To moderate phytotoxic injury of the antibiotics to cabbage and broccoli seedlings, seed treated with antibiotic and air-dried for 24 hours was soaked for 30 minutes in a specific salt solution (0.25*M*) and allowed to dry. The seed was then planted in sand and grown under a continuous light of 600 to 650 ft-c supplied by fluorescent lighting. Readings of cotyledonary chlorosis (yellow, whitish, purple discoloration) were made 6-7 days after seedling emergence and ratings made as follows: ++++ = very severe, cotyledons entirely chlorotic; +++ = severe, cotyledons chlorotic, 1 entirely and 1 partially; ++ = moderate, chlorosis partial on both cotyledons but distinct; + = slight, chlorosis marginal on 1 or both cotyledons.

EXPERIMENTAL RESULTS.—*Inhibitory levels of antibiotics and mercuric chloride on the pathogen.*—Inhibition of *X. campestris* by 5 antibiotics and mercuric chloride was determined at concentation levels ranging from 10-4,000 ppm (Table 1). In general a point was reached at concentrations of approximately 2,800-3,200 ppm where no further increase in inhibition occurred. The data indicate that the tetracyclines and mercuric chloride possess stronger *in vitro* inhibitory qualities than streptomycin and Agri-mycin.

Antibiotic seed treatment of naturally infected cabbage and broccoli seed.—Preliminary studies were made with various antibiotic seed treatments for periods of 5, 10, 15, and 20 minutes at concentrations ranging from 100-800 ppm. Complete seed disinfection was not obtained; however, seed treatments were more effective at the higher concentrations and with the longer treatments. Thus, a 30-minute seed treatment was decided upon for treatments with concentrations up to 3,000 ppm. Concentrations beyond 3,000 ppm were not used for seed treatments since 3,000 ppm is the level of maximum effectiveness (Table 1). Increased dosage above that concentration would, therefore, not be expected to increase the antibacterial effect. The number and percentage of seed infections after seed treatment with streptomycin, Terramycin, Aureomycin, and Achromycin are given in Table 2. A decrease in number of bacterial colonies occurred with increased concentrations of all antibacterial agents used and seed infection was reduced to less than 1%. At 3,000 ppm in *in vitro* experiments, Achromycin and streptomycin did not provide complete seed disinfection, whereas Aureomycin and Terramycin treatments appeared to provide complete disinfection. Agri-mycin containing 15% streptomycin and 1.5% Terramycin was no more effective in seed disinfection than either alone. Although seed infection was reduced to 0.05% with mercuric chloride at 1,000 ppm, its use was discontinued due to its high concentration, which renders it unsafe for general use.

Repeated seed treatment trials with Aureomycin at 2,400 and 3,000 ppm and with Terramycin at 3,000 ppm continued to provide complete seed disinfection. Considering the possibility of internally borne bacteria, seed treated at the same high concentrations was halved and plated. Out of 800 seed pieces plated, 2-3 pieces from seed treated with Aureomycin developed bacterial colonies 10 days after plating, whereas no colonies appeared from seed treated with Terramycin at 3,000 ppm.

Seed treated with Terramycin at 3,000 ppm was grown under environmental conditions favorable for disease development described later in this paper. In each of 3 replications of 200 seedlings each, 1 seed-

TABLE 1. Inhibition of *Xanthomonas campestris* by various antibiotics as determined by paper disc-agar diffusion method

Antibiotic concentration (ppm)	Diameter[a] of inhibition zones (mm)					
	Aureomycin	Achromycin	Terramycin	Streptomycin	Agri-mycin	$HgCl_2$
10	16	0	0	0	0	20
100	27	24	23	16	0	29
200	30	26	26	18	15	31
400	32	29	30	20	17	33
800	33	31	33	23	20	35
1200	34	32	34	24	22	36
1800	35	33	35	27	24	38
2400	36	34	36	28	25	38
2800	36	36	37	28	26	38
3200	37	36	38	29	26	38
3600	37	36	38	29	26	39
4000	37	36	38	29	26	39

[a] Average diameter of 4 replicates.

TABLE 2. Disinfection of black rot-infected seed by a 30-minute seed treatment with antibiotic solutions

Antibiotic concentrations	No. and % seed infections[a]							
	Streptomycin		Terramycin		Aureomycin		Achromycin	
	No.	%	No.	%	No.	%	No.	%
Untreated check	16	4.00	22	5.50	33	8.25	33	8.25
100 ppm	8	2.00	18	4.50	24	6.00	28	7.00
200 ppm	8	2.00	16	4.00	21	5.25	23	5.75
400 ppm	2	0.50	10	2.25	6	1.50	13	3.25
600 ppm	2	0.50	8	2.00	3	0.75	9	2.25
800 ppm	1	0.25	2	0.50	1	0.25	6	1.50
1000 ppm	1	0.25	1	0.25	1	0.25	2	0.50
1200 ppm	2	0.50	1	0.25	1	0.25	2	0.50
1800 ppm	1	0.25	3	0.75	2	0.50	2	0.50
2400 ppm	2	0.50	2	0.50	0	0.00	2	0.50
3000 ppm	1	0.25	0	0.00	0	0.00	2	0.50

[a] Based on 4 replicates of 100 seeds each.

ling developed black rot symptoms, indicating that Terramycin does not completely disinfect seed at concentrations as high as 3,000 ppm. Similar assay of seedlings produced from seed treated with Aureomycin, Achromycin, and streptomycin at 3,000 ppm showed disinfection to less than 1%.

Assay of treated seed for antibiotic absorption.—Antibiotics at concentrations ranging from 10-2,400 ppm failed to penetrate the seed coat of cabbage and broccoli seed when applied as seed treatments. Zones of inhibition occurred around whole seeds and halved seed coats, and increased in diameter with increased concentration. Inhibition zones were not visible around the halved seed pieces from which the seed coats had been removed, but a concentrated growth of the test organism around the internal seed parts was noted in assays for streptomycin and Terramycin, which indicates some antibiotic might have been absorbed. At low concentration levels, the antibiotic has no inhibitory effect and may actually stimulate growth of susceptible organisms. Seed treatments of 2-4 hours enhanced absorption but resulted in rupture of the seed coat. Treatments of 30-minute and 1-hour periods under vacuum, and Tween 20, 1% glycerine, acetic acid, and alcohol added to antibiotic solutions and in pretreatments had no favorable effect on absorption.

Antibiotic seed treatments of artificially inoculated cabbage seed.—The foregoing experiments did not make clear whether the antibiotics are bactericidal or bacteriostatic. Treatment of artifically inoculated seed with the antibiotics at 3,000 ppm reduced bacterial colonies to less than 8% compared with 93% in the control. The appearance of bacterial colonies on control plates was complete within 3-4 days after plating, whereas colony development was delayed from seed treated with the tetracyclines until 9-10 days after plating and continued to appear until 18 days after plating. Apparently, the effect of the tetracyclines on the bacteria is largely bactericidal but bacteriostatic to a low percentage of the bacterial population. Colonies began to appear from seed treated with streptomycin on the same day as in the control plates and continued for 7 days, after which time new colonies did not develop. Results indicate bactericidal action of streptomycin with the exception of a small percentage of the bacteria which appear to be resistant to the antibiotic.

Resistance of X. campestris to streptomycin sulfate.—A 3,000-ppm concentration of each antibiotic was made by dissolving the antibiotic in agar and pouring in petri plates. Each plate was inoculated with 0.1-0.5 ml of a heavy bacterial broth after the agar solidified. After 5 days' incubation at 28°C, bacterial colonies appeared in the streptomycin-agar plates, but no bacterial growth occurred over a 4-week period in agar plates containing Aureomycin, Terramycin, and Achromycin. Transfers of bacteria were made from streptomycin plates to nutrient agar plates and these in turn were transferred to fresh agar plates until 6 successive transfers were made. When bacterial cultures grown from the 6th transfer were assayed, the bacteria were inhibited by the tetracyclines at 3,000 ppm but not by streptomycin. Typical black rot symptoms developed on cabbage plants inoculated with the streptomycin resistant bacteria, and upon reisolation the bacteria were still resistant to the antibiotic. Since bacteria from a fresh isolation were seeded on the agar plates containing separate antibiotics, the possibility of acquired resistance due to previous antibiotic exposure is unlikely. Therefore, growth of bacteria on the streptomycin plates apparently resulted from organisms which inherited a resistant metabolic state prior to contact with the antibiotic. Since the bacteria remained resistant to streptomycin after several transfers on agar and passage through cabbage plants, the resistance was passed on to the resulting population growing from cells which were initially resistant or had become more resistant.

Effect of combined antibiotics on X. campestris.—Antibiotic combinations, at a concentration of 3,000 ppm each, provided an increase in inhibition compared to inhibition by the single components (Table 3). Seed treatments of artificially inoculated seed were made with antibiotics combined in solution and also by successive treatment with 2 antibiotics. Combinations of tetracyclines, streptomycin-Aureomycin, and streptomycin-Achromycin delayed growth of bacterial colonies from the treated seed until the 16th day after plating, and reduced the number of colonies to less than 5% compared with 8% after treatment with individual antibiotics. The early occurrence of bacterial colonies from streptomycin-treated seed was eliminated with all tetracycline-streptomycin combinations. Successive treatment of seed samples with 2 antibiotics did not effectively increase disinfestation of contaminated seed compared with the combination treatments. Resistance of the bacteria to streptomycin may have increased their sensitivity to the other antibiotic

TABLE 3. Inhibition of *Xanthomonas campestris* by antibiotic combinations as determined by paper disc-agar diffusion method

Antibiotic combinations (3,000 ppm)	Diameter[a] of inhibition zones (mm)				
	Combinations	Terramycin	Aureomycin	Streptomycin	Achromycin
Streptomycin + Terramycin	42	40		32	
Streptomycin + Aureomycin	39		38	31	
Streptomycin + Achromycin	41			32	39
Terramycin + Achromycin	44	38			41
Terramycin + Aureomycin	44	40	43		
Aureomycin + Achromycin	45		41		43

[a] Average diameter of 4 replicates.

in combination, thereby eradicating the bacteria from which the early appearing colonies develop. According to Goldberg (4), resistance to one antibiotic may make an organism more sensitive to another. Reduction in the number of colonies developing from treated seeds may be due to an interaction of the antibiotics whereby an increase in activity would result in death of bacteria otherwise inhibited temporarily by individual antibiotics.

Effect of antibiotic sprays on disease development in cabbage seedlings.—Assays of plant sap extracted from cotyledons of cabbage seedlings sprayed with antibiotics at a concentration of 400 ppm showed that Terramycin, Aureomycin, streptomycin, and Achromycin were absorbed by cabbage seedlings. Terramycin, streptomycin, and Achromycin sprays at 1,000 ppm plus 1% Na-K-chlorophyllin (1) solution were applied on young cabbage plants prior to and following inoculation with black rot bacteria. In 2 trials, lots of 40 plants sprayed with individual antibiotics 2 and 3 times after inoculation were infected comparably to the controls. In a 3rd trial, plants sprayed 1 day prior to inoculation and again 2 days after inoculation, the percentage of infected plants was 9, 63, and 69%, respectively, with streptomycin, Achromycin and Terramycin sprays, compared with 97% in untreated controls. The same sprays applied to cabbage seedlings grown from seed treated for 30 minutes with respective antibiotics at 3,000 ppm did not eradicate black rot bacteria viable after seed treatments and approximately 2% seedling infection occurred. Where sprays were applied to cabbage seedlings grown from naturally infected seed 12 hours prior to moisture chamber exposure, the percentage of infected plants was 15, 4, and 12% respectively for Terramycin, streptomycin, and Achromycin, compared with 16, 11, and 19% in lots sprayed 12 hours after moisture chamber treatment. In control lots 20% seedling infection occurred. Marked reduction of disease incidence was provided by the streptomycin sprays. The sprays apparently serve as protectants, hindering plant infection by the bacteria.

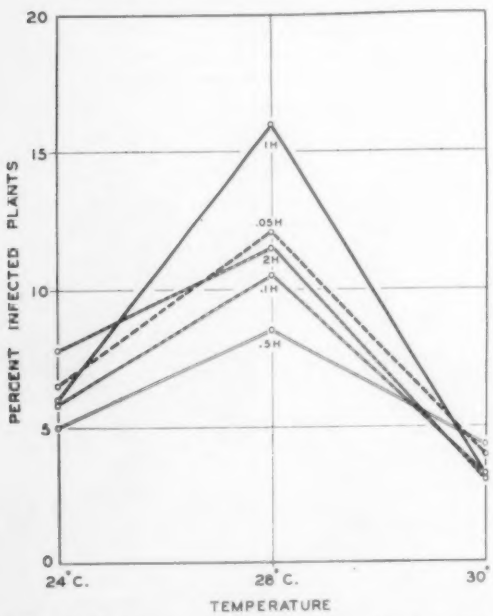

Fig. 1. Development of black rot on cabbage seedlings grown in sand-vermiculite at 3 different temperatures and supplied with balanced nutrient solutions.

Seedling assay for presence of black rot infection in crucifer seed samples.—Conditions studied for maximum symptom development on cabbage seedlings grown from infected seed included temperature, nutrient solution levels, photoperiod, preplanting treatment, and moisture. Fig. 1 represents the average percentage of infected plants of 3 replicate nutrient and temperature studies with 200 seedlings/replicate. Disease development was markedly enhanced at 28°C. The differences in disease incidence at the various nutrient levels at 24° and 30°C were not marked. However, at 28°C maximum disease incidence of 16% occurred at the 1H level and decreased to 11.5% at 2H and 7.5% at 3H (not shown). It seemed logical to expect a gradient of severity, increasing or decreasing with nutrient concentration, but this was not the case.

Seedlings were observed for symptom development during a 14-day period following transfer from the moisture chamber to the growth chamber. At 28°C the 1st symptoms appeared 6 days after transfer from the moisture chamber compared with 8 or 9 days at 24° and 30°C. Since symptom appearance on seedlings grown at different nutrient levels at a specific temperature began on the same day, the influence on rapid disease development can be attributed to temperaure rather than nutrient levels.

A period of at least 6-12 hours in a moisture chamber was found to enhance considerably seedling infection. In each of 2 lots, 200 plants were placed in a moisture chamber for periods as follows: lot 1, 6 hours; lot 2, 12 hours; lot 3, no exposure in a moisture chamber. During a 14-day period the average % of infected seedlings in 3 replications showing visible symptoms was 9 in lot 1, 12 in lot 2, and 4 in lot 3. The moisture chamber treatment apparently provides a means by which bacteria contaminating the cotyledons can reach the margins and become established.

The influence of photoperiod on disease development is shown by data given in Table 4. Nutrients were supplied to both the 12- and 24-hour-day plants with 0.1H solution. In addition to a higher percentage of infected seedlings in the 24-hour-day seedlings, symptoms appeared 2 days earlier than in the 12-hour-day plants. Thus, the length of photoperiod influences disease development as does temperature. Since the xylem-invading black rot bacteria grow well when nonorganic nitrogen is supplied, they would directly benefit from conditions conducive to photosynthesis. Accumulation of sugars and starch in long-day plants would further enhance growth of bacteria breaking

TABLE 4. Influence of photoperiod upon development of black rot symptoms on cabbage seedlings grown from infested seed

Replicates[a]	Plants visibly infected: 24-hr day %	Plants visibly infected: 12-hr day %
	28	12
1	24	10
	24	12
	46	8
2	32	16
	36	6
	26	10
3	24	8
	34	12

[a] Each replicate represented by 3 pots with 50 plants/pot.

out of the xylem into surrounding tissues and utilizing available food.

An increase in disease incidence was provided by soaking infected seed in distilled water at 28°C for periods of 2, 4, 8, and 12 hours prior to planting. An average of 3 trials showed 17, 23, 39, and 18% infected seedlings, respectively, from seed samples soaked for 2. 4, 8, and 12 hours, compared with 13% infected seedlings grown from nonsoaked seed samples. Soaking the seed apparently enhances bacterial multiplication and spreading among seeds, thereby increasing the chances of seedling infection. The percentages of infected plants for the different periods of treatment represent a curve resembling a bacterial growth curve with a peak reached in these experiments at approximately 8 hours and a period of death and decline after 8 hours.

To test the reliability of the seedling assay, seed samples were obtained from Europe and several states in the United States and planted under optimal conditions for disease development. During a 14-day period, 6 of 27 samples showed black rot infection.

Phytotoxic effects of antibiotics on cabbage and broccoli seedlings grown from treated seed.—Germination of cabbage and broccoli seed was not affected by 30-minute seed treatments with antibiotic solutions at 200 and 1,000 ppm concentrations. Treatment with mercuric chloride solution, however, reduced germination approximately 5% at 200 ppm and 25% at 1,000 ppm. Root and stem measurements of seedlings produced from seed treated at 600 ppm showed a marked inhibition of growth by streptomycin and to a lesser extent by Terramycin, Aureomycin, and Agri-mycin.

A general chlorosis and stunting of seedlings in the cotyledonary stage was caused by the antibiotics used and increased in severity with increased concentrations and length of treatment. Cotyledons on broccoli were yellow and in severe cases whitish; on cabbage seedlings a purple discoloration also occurred. Slight chlorosis became evident on seedlings grown from seeds treated with Terramycin and Achromycin solutions at 150 ppm. With treatments at 50 ppm, slight chlorosis is caused by Aureomycin and slight to severe chlorosis by streptomycin. Affected seedlings generally recovered to a normal green color, with slightly affected plants recovering more rapidly than those severely affected. Seedlings with severely bleached cotyledons usually died without regaining normal color. Rosen (6) investigated the phenomenon of chloroplast bleaching in higher plants and observed that the toxic effect is not on the photosynthetic mechanism alone but also inhibits growth of the plants. Apparently, streptomycin suppresses chlorophyll development in higher plants but does not affect chloroplasts after they are formed, thus interfering with their synthesis rather than their function. Since chlorosis was commonly caused by the antibiotics used in these experiments on cabbage and broccoli seedlings, it is assumed the antibiotics may have interfered with chlorophyll synthesis.

Chlorosis was less evident on broccoli seedlings grown in muck soil than on seedlings grown in sand. A 2- to 3-minute rinse with distilled water immediately after seed treatment did not have an effect on the degree of chlorosis. A 30-minute water wash, however, immediately following treatment of seed with antibiotic considerably reduced the intensity of chlorosis. Phytotoxic injury also was eliminated by removing seed coats from seed treated with Aureomycin, streptomycin, Achromycin, and Terramycin.

Several chemical compounds were selected for use as anti-phytotoxic treatments and applied as 30-minute treatments to broccoli seed previously treated with an antibiotic. Seedlings were grown in sand with a 24-hour photoperiod of 600-650 ft-c at approximately 25°C and maintained with a 1H solution. Data (Table 5) show the effect of various chemical compounds on reduction of phytotoxic injury to broccoli seedlings caused by Achromycin. Sodium and potassium phosphate had a marked effect in reducing toxic injury (both chlorosis and stunting), caused by Aureomycin, Achromycin, and Terramycin (Fig. 2) at 1,000 ppm concentrations. Calcium, potassium, and sodium

TABLE 5. Effect of various chemical solutions applied as safeners for reduction of phytotoxicity to cabbage seedlings grown from seeds treated with Achromycin at a concentration of 1,000 ppm

Treatments[a]	No. of chlorotic plants[b]	Intensity of chlorosis[c]	% of reduction	Dead seedlings
Antibiotic check	96	++++	—	15
KH_2PO_4	8	++	92	7
NaH_2PO_4	7	++	93	15
$CaCl_2$	9	++	91	7
KCl	8	++	92	7
NaCl	7	++	93	10
Na-K-chlorophyllin[d]	75	++++	22	8
Untreated check	0			3

[a] Treatments with chemical solutions of (0.25*M*) applied to seed after treating with antibiotic and drying.

[b] Average of 3 replicates with 100 plants/replicate.

[c] Intensity of chlorosis is rated as follows: ++++ = very severe; +++ = severe; ++ = moderate; and + = slight.

[d] 1% concentration.

Fig. 2. Reduction of phytotoxicity on broccoli seedlings grown from seeds treated for 30 minutes with 1,000 ppm Terramycin, by post-treatment with a (0.25*M*) sodium phosphate solution. Seedlings were grown from untreated seed (left), seed treated with Terramycin (center), and seed treated with Terramycin followed by treatment with a sodium phosphate solution (right).

chloride were effective against Achromycin and Terramycin injury but less effective against Aureomycin. Only calcium chloride treatment reduced toxicity caused by streptomycin. Solutions of 0.1*M* and 0.05*M* were not as effective in moderating phytotoxic injury as 0.25*M* and 0.5*M* solutions, and 30-minute treatments were more effective than treatments of 2, 5, 10, or 15 minutes. Since toxic injury seems to occur from antibiotics adsorbed on the seed coat, the action of the salt solutions in reducing toxicity may be partially due to washing antibiotic from the seed coat. The pH differences between the antibiotic solutions and the salt solutions may also be a factor influencing toxicity reduction. Assays and seed treatment studies showed that calcium chloride had an adverse effect on antibacterial activity of the tetracyclines and streptomycin. Potassium and sodium phosphate also reduced the activity of streptomycin but had no adverse effect on activity of the tetracyclines.

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SEVERITY AND DISTRIBUTION OF COTTON BOLL ROTS AS RELATED TO TEMPERATURE

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SUMMARY

Several cotton boll-rotting fungi were separated into low- and high-temperature groups according to growth in artificial culture and boll rot development. The optimal temperatures for rotting of cotton bolls by these fungi (24° and 33°C) coincided with optimal temperatures for fungus growth on PDA. Surveys in California showed that the low-temperature boll rot *Nigrospora oryzae* was more prevalent in the cooler central valley (summer avg. 26°C), whereas the high-temperature boll rots *Aspergillus flavus*, *A. niger*, and *Rhizopus nigricans* were more widespread in the hotter southern valleys (summer avg. 32°C). The results indicated that the temperature requirements for fungus growth and boll rot development were related to the severity and distribution of the major boll rots in California and the high incidence of bright greenish-yellow fluorescence caused by *A. flavus* in western-grown cotton.

INTRODUCTION.—Surveys of cotton (*Gossypium hirsutum* L.) diseases in California during 1957-60 have revealed a number of distinct boll rot diseases that vary both in severity and distribution throughout the cotton-growing areas of the state. The major boll rot fungus in the central valley (San Joaquin) is *Nigrospora oryzae* (Berk. & Br.) Petch. which was found in 94% of the diseased bolls examined. Only 1-2% of the rotted bolls were infected by *Aspergillus flavus* Lk. ex Fr. and *A. niger* v. Tiegh. The latter 2, however, were the most prevalent fungi in southern California (Palo Verde and Imperial Valleys), occurring on 68% of the diseased bolls examined. *N. oryzae*, in contrast, was found in only 12% of the rotted bolls there. Found in trace amounts in both regions were *Fusarium moniliforme* Sheldon and *Rhizopus nigricans* Ehr.

Several distinct boll rots are described in the literature. In 1927 Shapovalov (14) reported that the most common fungi on decayed bolls in the southwestern United States were *A. niger* and *R. nigricans*. Bollenbacher and Marsh (1) and Marsh and Taylor (7) have shown that yellow stain of cotton lint which produces a bright greenish-yellow (BGY) fluorescence under ultraviolet light is associated with *A. flavus*. This disease has been found primarily in cotton from California, Arizona, and Texas (1, 7). More recently a new boll rot in California caused by *N. oryzae* was described by Houston and Garber (4). Another boll rot, caused by *F. moniliforme* and frequently encountered in California, was described by Woodroof (16). Studies (2, 10, 15) in the eastern United States indicate that the important boll rot pathogens there are *Glomerella gossypii* Edg., *Alternaria* spp., *Diplodia gossypina* Cooke, and *Xanthomonas malvacearum* (E. F. Smith) Dowson. In the western United States, in contrast, *Glomerella* boll rot is not present; and *Alternaria* and *Diplodia* are of minor importance only. *X. malvacearum* also occurs; but, according to Schnathorst, Halisky, and Martin (13) the boll rot phase of the disease is not severe. Thus, the major boll-rotting organisms in California differ from those in the eastern United States.

The present study tests the hypothesis that differences in the severity and distribution of major boll rots in California are related to temperature. Experiments were conducted: 1) to determine the relation of temperature to fungal growth in culture, and 2) to determine the effect of temperature on the development of boll rot diseases. The results were then related to summer temperatures prevailing in the cotton-growing regions of the state and to their apparent influence on distribution of the diseases.

RESULTS.—*Temperature requirements of boll-rotting fungi.*—Pure cultures of boll-rotting fungi were obtained from field collections of diseased bolls and their growth rates determined on potato-dextrose agar (PDA), about pH 7, at temperatures ranging from 12-39°C at 3° intervals. PDA plates were inoculated with 5-mm discs of mycelia of the various fungi, except the *Aspergilli*. With those fungi, the inoculum was prepared by suspending spores in melted agar at about 45°C, permitting the agar to solidify, and transferring 5-mm discs of the suspended spores to PDA plates. Each inoculum disc was placed in the center of the plate, and growth rate was determined by measuring colony diameters after various periods. There were 6 replications of each fungus-temperature combination.

The results indicate that these fungi can be separated into low- and high-temperature groups (Fig. 1). The fungi in the low-temperature group, *F. moniliforme* and *N. oryzae*, grew at 12-33°C, with the optimum at 24°C. *N. oryzae* grew faster (1.1 mm/hr at optimum) than did *F. moniliforme* (0.73 mm/hr). The fungi in the high-temperature group, *A. niger*, *A. flavus*, and *R. nigricans*, did not grow at 12°C, grew best at 33°C, grew well at 36°C, and only slightly at 39°C (Fig. 1). *R. nigricans* had a narrower growth range (18-39°C) than the 2 *Aspergillus* species (15-39°C). At 33°C, *A. niger* and *A. flavus* grew at rates of 0.6 and 0.65 mm/hr, respectively; whereas *R. nigricans* grew much more rapidly (2.7 mm/hr).

Temperature requirements for boll rot development.—The effect of temperature on boll rot development was studied in the laboratory. Bolls of Acala 4-42

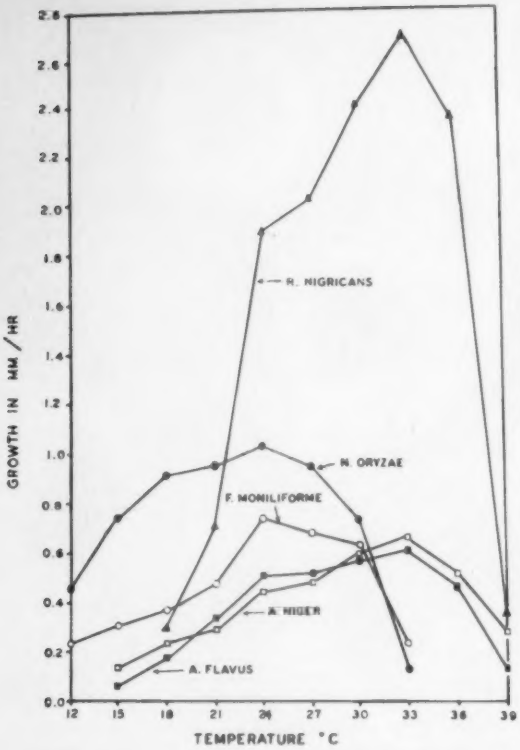

Fig. 1. Comparative growth rates (mm/hr) of 5 boll-rotting fungi incubated on PDA at 10 constant temperatures.

cotton were collected from the field at mature size prior to opening. They were dipped 30 seconds in 75% ethyl alcohol, 4 minutes in 1:1,000 mercuric chloride, and rinsed in sterile distilled water. Each boll was cracked open at the tip with a sterile scalpel and inoculated with mycelia of the various fungi borne on small pieces of agar. The inoculated boll was placed in a sterile 100-ml beaker containing about 20 ml of distilled water. The beaker was then placed in a sterile 1-qt jar with a cotton plug similar to that used by Marsh, *et al.* (6). The space between the beaker and the wall of the jar was filled with a pad of moistened cotton (Fig. 2).

Incubation was at 21°, 24°, 27°, 30°, and 33°C. There were 5 replications of each fungus-temperature combination. Degree of internal and external decay, as indicated by loss of tensile strength, pigmentation, and general degradation of fiber quality, is indicated on a scale of 0-4, from no rot to severe rot (Fig. 3).

The results indicate 2 distinct groups (Table 1): 1) lower-temperature (*F. moniliforme* and *N. oryzae*), with boll rot optimum at 24°C; and 2) higher-temperature (*A. flavus, A. niger, R. nigricans*), with rot optimum at 33°C. *N. oryzae* was the only fungus that rotted bolls internally (Fig. 4) without extensive external damage. *R. nigricans* rotted bolls faster than any of the other fungi, followed by *N. oryzae, A. niger, A. flavus,* and *F. moniliforme* (Table 1). In general, growth rate on PDA was directly correlated with rate of rotting development, and the optimal temperature for fungus growth (24° and 33°C) coincided with the optimal temperature for the rotting of bolls by the respective fungi.

Discussion and Conclusions.—A study of the etiology of boll rots of cotton implicates several species of fungi as pathogenic agents. In the United States boll rots are coextensive with the cultivated cotton crop. The pathogenic organisms associated with major boll rots of eastern- and western-grown cotton, however, are not identical. This disparity in the geographic distribution of boll rot organisms is indicative of the paramount importance of environmental factors in the development and distribution of boll rot diseases.

In a major contribution to the ecology of cotton boll rots, Miller and Weindling (10) noted the wide distribution and frequency of cotton anthracnose (*Glo-*

Fig. 2. Sterile incubation chamber used in temperature studies with boll rot diseases: a 100-ml beaker containing distilled water, a moistened cotton pad for maintaining atmospheric humidity, and an incubated cotton boll overgrown with *Aspergillus niger.*

merella boll rot) in 11 states east of Texas and Oklahoma, but an apparent absence of the disease westward. They observed a correlation between disease distribution and Thornwaite's classification of climate zones based on precipitation effectiveness. Miller (8, 9) substantiated these observations in a study of the effect of humidity on the survival and sporulation of the anthracnose fungus, attributing its absence in western cotton to unfavorably dry conditions that prevent spore survival in cotton fields.

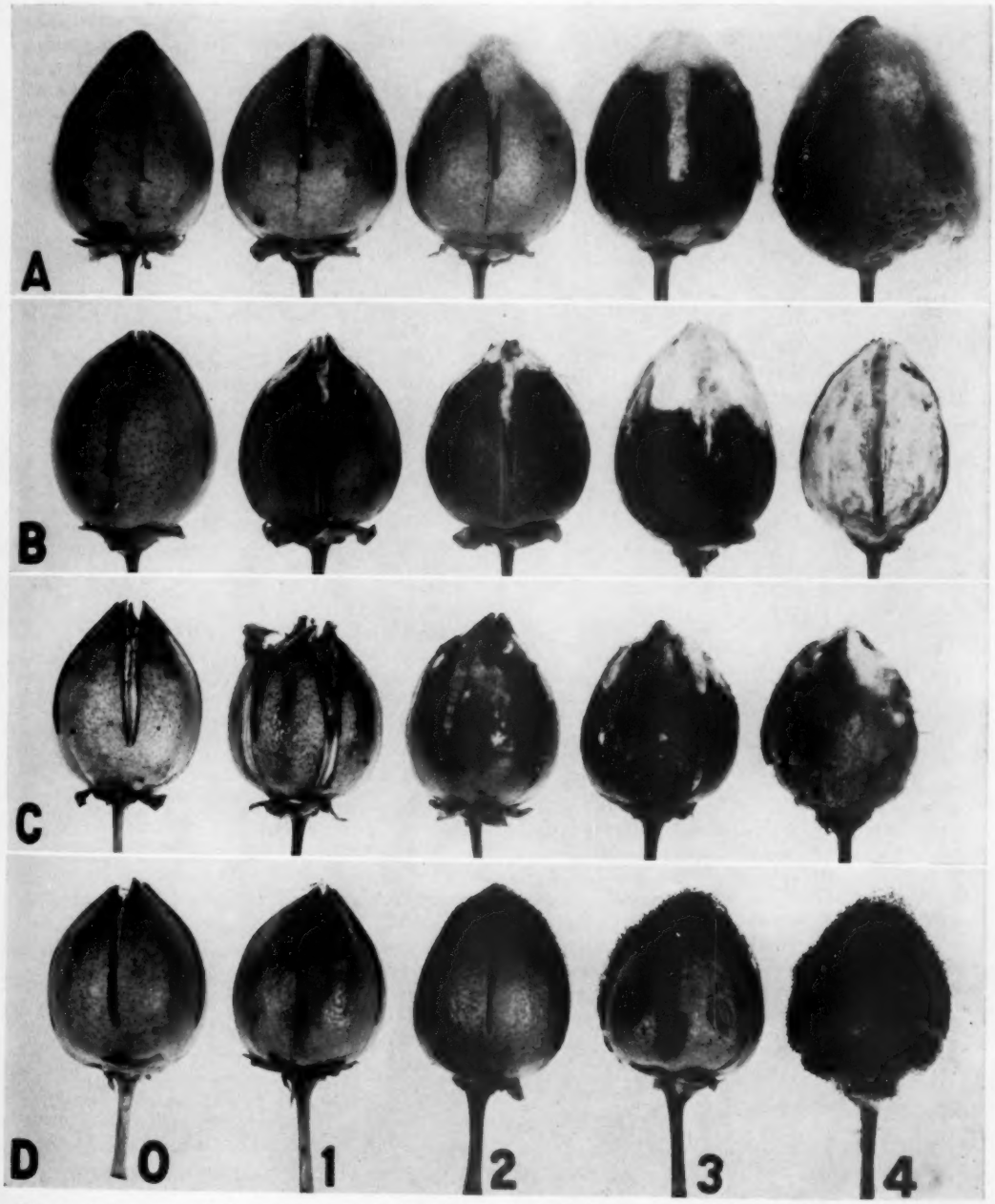

Fig. 3. Four common boll rots of cotton showing (top to bottom) diseases caused by **A)** *Rhizopus nigricans*, **B)** *Fusarium moniliforme*, **C)** *Aspergillus flavus*, and **D)** *A. niger*. The scale (0-4) indicates rotting severity as measured by both surface sporulation of fungi and decomposition of cotton bolls.

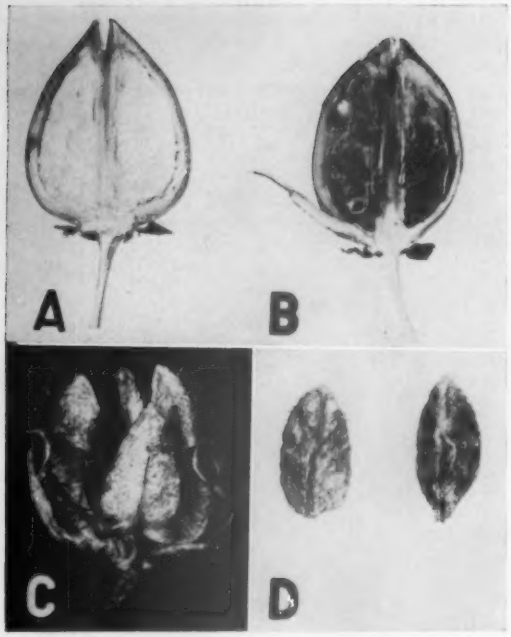

Fig. 4. Internal boll rot of cotton caused by *Nigrospora oryzae*: **A)** cross section of a healthy boll, **B)** cross section of a diseased boll, **C)** mature boll with the tight-lock symptom of the disease, and **D)** diseased locks which remain unfluffed and readily drop to the ground.

Bollenbacher and Marsh (1) and Marsh and Taylor (7) showed that the BGY fluorescence in cotton lint associated with *A. flavus* was found primarily in western-grown cotton. Schnathorst (12) found that 30-33°C was more favorable for production of the BGY pigment by *A. flavus* than 24-27°C and suggested that the high incidence of yellow stain of cotton lint in the southwestern United States was related to high summer temperatures. Marsh and Taylor (7) pointed out that areas producing fluorescent cotton also were characterized by exceptionally high temperatures when the cotton bolls were opening. In California, *A. flavus* infects raw cotton fibers when relative humidity and temperatures are high under rank cotton foliage (3). The rank growth associated with exceptionally high temperatures and high humidity creates conditions favorable for boll rotting. These factors contribute to the severity and prevalence of cotton boll rots in the southern valleys of California.

Kimball and Brooks (5) recently illustrated the plant climate zones for California, showing effective day and night monthly temperatures for the respective zones. Their data indicate that the average summer temperature is 26°C in the central San Joaquin Valley, and about 32°C in the southern Coachella, Palo Verde, and Imperial valleys. These 2 areas encompass the major cotton-producing valleys of California (Fig. 5). The present studies indicate a direct relationship of temperature to the geographic distribution of boll rots in the plant climate zones of the state. The experimental evidence suggests that *A. flavus* and *A. niger* are more widespread in southern California because the higher summer temperatures favor their development. Furthermore, boll rotting by *A. flavus* is favored by exceptionally high incubation temperatures (Table 1), which would account in part for the high incidence of yellow stain in southern California, Arizona, and Texas. Similarly, the data also suggest that boll rotting by *N. oryzae* is more severe in the central valley of California because lower summer temperatures favor its development. Thus, the temperature requirements for fungus growth and boll rot development explain in part the severity and distribution of the major boll rots in California and the high incidence of yellow stain in western cotton.

Distribution of the minor boll rots is influenced by modifying factors. Black boll rot due to *R. nigricans* is associated most frequently with insect injury, particularly by the common boll worm (11, 14). Once the organism gains entry, it destroys the bolls more rapidly and completely than any other organism studied (Table 1). Pink boll rot due to *F. moniliforme* is more difficult to evaluate since it is often found (10% of diseased bolls) in the warmer southern valleys of California, where prevailing temperatures appear somewhat unfavorable for its growth. Thus, some factors other than optimal temperature influence pink boll-rot distribution. *F. moniliforme* is generally considered a weak pathogen, and other workers (8, 11) have noted that infections occurred where the boll

Fig. 5. Prevailing average summer temperatures and major boll-rotting fungi in the 2 cotton-growing areas of California.

TABLE 1. Differences in severity of rotting of Acala 4-42 cotton bolls by 5 fungi at different incubation times and temperatures

Temperature (°C)	Fungi and severity of boll rotting[a]				
	F. moniliforme	*N. oryzae*	*A. flavus*	*A. niger*	*R. nigricans*
21	2.4	2.8	1.0	1.4	1.2
24	3.4	4.0[b]	2.0	1.4	2.0
27	1.8	4.0[b]	2.2	2.4	2.4
30	2.2	2.4	4.0	2.4	3.6
33	2.6	1.2	4.0	3.6	4.0
Incubation (hr)	184	144	160	144	92

[a] Severity of rotting of bolls was determined by the degree of both fiber and boll deterioration. Degree of rotting was measured on a scale of 0-4 (Fig 3). The numbers in the table are an average of 5 replicates at each fungus-temperature combination.

[b] Severity of boll rotting and fiber deterioration by *N. oryzae* was more pronounced at 24°C than at 27°C, although both temperatures were conducive to severe rotting as indicated by the maximum grade (4.0) assigned.

was punctured or otherwise mechanically injured, thus relegating its role to that of a secondary invader.

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POTENTIAL PATHOGENICITY OF PYTHIUM ISOLATES FROM FOREST NURSERIES

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SUMMARY

Pathogenicity of 27 *Pythium* isolates, mainly *P. debaryanum* and *P. ultimum*, was tested on aseptically germinated seedlings of 29 tree species and vegetable cultivars. None of the isolate-host combinations showed marked specificity. Different hosts, however, ranked in susceptibility from highly susceptible to most of the isolates, to resistant to most of the isolates; different isolates ranked in pathogenicity from highly pathogenic to most of the hosts, to highly pathogenic to none of the hosts. It is suggested that some of the highly susceptible plants, such as West India gherkin, could be used as bait in studies on soils containing *Pythium*. Plants with intermediate susceptibility are more suitable for comparing relative pathogenicity.

INTRODUCTION.—Over a period of 7 years a large number of *Pythium* isolates have been obtained from damped-off tree seedlings from forest nurseries in Saskatchewan and Manitoba. These isolates have been maintained in tube slants at low temperatures under paraffin oil. Tests of potential pathogenicity have been made on a few tree species with a large number of fungi including some *Pythium* (9) and *Phytophthora* (8, 9) isolates. Marked pathogenicity was demonstrated for all isolates of the 4 *Pythium* species and of the 1 *Phytophthora* species tested. A progeny of *Picea glauca* (Moench) Voss that was fairly resistant to an isolate of *Pellicularia praticola* (Kotila) Flentje was highly susceptible to a *Pythium* isolate (9).

In exploring the importance of different isolates farther, the potential pathogenicity of additional isolates was tested on a number of different hosts, using a modification of a culture tube method described earlier (9). In addition to tree species, an assortment of vegetable cultivars was utilized. It was presumed that the continued availability and greater genetic homogeneity of the vegetable cultivars would be an advantage in standardizing tests of damping-off pathogens.

MATERIALS AND METHODS.—Seeds were surface-sterilized with $HgCl_2$ in 50% alcohol, rinsed, and planted on malt agar in petri dishes. Contaminated seeds were soon detected and discarded.

Tubes one-fourth full of slightly acidified Difco cornmeal agar (pH 5.9) were inoculated with *Pythium* isolates and closed with inverted glass vials. When mycelium had covered the agar surface, 5 seeds in the same early stage of germination were placed in each tube.

The tubes were incubated in growth chambers while immersed in water baths to the depth of the agar necessary to control the temperature of the medium. The tubes were exposed to continuous light (1,500 ft-c) from cool white fluorescent tubes. The tests were made in 3 series, each at a slightly different temperature between 16° and 22°C.

Of the possible 812 combinations of 29 hosts (listed under results) and 28 fungus isolates, 417 were tested, each with 2 or more replications. Ten of the isolates were identified as *P. debaryanum* Hesse, 6 as *P. ultimum* Trow, 1 as *P. periplocum* Drechs., and 1 as *P. acanthicum* Drechs. Eight of the 10 other isolates resembled *P. debaryanum* or *P. ultimum*, but produced the sexual stage so seldom as to preclude positive identification. An isolate of *Phytophthora cactorum* (Leb. & Cohn) Schroet., similar to the 1 previously tested (8) on a number of host species, was used for comparison of pathogenicity. (Identification of *Pythium* species was made by Dr. J. T. Middleton, University of California.)

RESULTS.—Typical mortality percentages of seedlings 5 weeks after germination and inoculation are shown in Table 1. In these results, some isolates were highly pathogenic to all except the few hosts that were generally resistant to all the isolates; some were moderately pathogenic to all except a few hosts; and others were not highly pathogenic to any of the hosts. All isolates of *P. debaryanum*, *P. ultimum*, and similar ones assumed to be *P. debaryanum* or *P. ultimum*, exhibited high or moderate pathogenicity. The isolate of *P. acanthicum*, the isolate of *P. periplocum*, and 2 unidentified isolates exhibited low pathogenicity. The isolate of *Phytophthora cactorum* was intermediate in pathogenicity between these 2 groups. On tree species, *P. cactorum* and all except the 4 weakly pathogenic isolates killed most of the seedlings over a period of 5 weeks.

The mortality data on which order of susceptibility was based (Table 1, plus data not listed) form a series descending by small differences from most susceptible to most resistant hosts. The approximate order of average susceptibility of 9 tree species and 20 vegetable cultivars to 7 *Phythium* isolates at 16°C (1-week tests) was as follows:

Susceptible (35-90% mortality)—West India gherkin (*Cucumis anguoria L.*), Riverside Sweet Spanish and Yellow Globe Danvers onions (*Allium cepa L.*), American elm (*Ulmus americana* L.), Early Yellow Globe onion, Mammoth Red Rock, Danish Ballhead and Penn. State Ballhead cabbages (*Brassica oleracea* var. *capitata* L.).

Moderately susceptible (16-30% mortality): White spruce (*Picea glauca* (Moench) Voss), Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco), Sitka spruce (*Picea sitchensis* (Bong.) Carr.), Alta Sweet rutabaga (*Brassica campestris* var. *napobrassica* (L.) DC.), Bounty tomato (*Lycopersicon esculentum* Mill.), Coreless Nantes carrot (*Daucus carota* L. var. *sativa* DC.), redwood (*Sequoia sempervirens* (D. Don) Endl.), Grand Rapids leaf lettuce (*Lactuca sativa* L.), Danvers Halflong carrot, Early Northern sugar beet (*Beta vulgaris* L.), Laurentian rutabaga, Golden rape

TABLE 1. Percentage mortality of 5-week-old seedlings in test tubes with cultures of 4 *Pythium* isolates (examples from 1 test series)

Host	Isolate No.[a]				
	2658	1083	1366	1938	Avg.
Ulmus americana	100	100	100	30	82
Pseudotsuga menziesii	100	90	90	40	80
Sequoia sempervirens	100	80	100	0	70
Thuja occidentalis	90	80	60	20	62
Picea glauca	100	70	80	0	62
Pinus banksiana	100	50	70	0	55
Caragana arborescens	90	50	40	0	45
Pinus sylvestris	90	50	20	10	43
Mammoth Red Rock cabbage	100	100	50	0	62
Danish Ballhead cabbage	100	100	50	0	62
Penn. State Ballhead cabbage	100	90	20	0	52
Golden rape	100	90	0	0	48
Coreless Nantes carrot	90	40	50	0	45
Great Lakes Improved lettuce	50	20	0	0	18
Sweetheart lettuce	0	30	30	0	15
Avg.	87	69	51	7	

[a] 1083, 1366, and 2658 = *P. debaryanum*; 1938 = *P. acanthicum*.

(*Brassica napus* L.), Purple Top turnip (*Brassica rapa* L.), Chantenay Redcored carrot.

Resistant (0-15% mortality): Great Lakes Improved lettuce, eastern white cedar (*Thuja occidentalis* L.), caragana (*Caragana arborescens* Lam.), jack pine (*Pinus banksiana* Lamb.), New York #12 lettuce, Scots pine (*Pinus sylvestris* L.), Sweetheart lettuce.

The average mortality for week-old seedlings was not significantly different between trees and vegetables. Average losses produced by 7 *Pythium* isolates and 1 *Phytophthora cactorum* isolate were 23% in 9 tree species, and 25% in 14 vegetable cultivars. On the other hand, while tree seedlings continued susceptible for 5 weeks, most of the vegetable seedlings became resistant much earlier. Four weeks after the above mortalities had been noted, the mortality in the tree seedlings had increased by an additional 40%; in the vegetable seedlings, the increase was only 9%.

There was no marked host-pathogen specificity, although the hosts showed consistent differences in general susceptibility, and the fungus isolates showed consistent differences in general pathogenicity. Although temperatures differed slightly among the 3 series of tests, this did not significantly affect the order of mortality among different hosts and by the different isolates.

DISCUSSION.—The prolonged susceptibility to damping-off found in the tree species tested here also has been the general experience in nursery practice, especially in conifer beds. On the other hand, the rapidly acquired resistance found in vegetables may be characteristic of many other plants. Halpin and Hanson (3) reported that several legumes are very susceptible to many *Pythium* species but only when very young, less than 3 days old.

The high pathogenicity of isolates of *P. debaryanum* and *P. ultimum* with little or no host-specificity is in accordance with the results of many authors on various tree species (1, 2, 4, 7, 10, 11) and on various agricultural plants (3, 5, 6). Eliason (1) found 4 conifer species susceptible to most of the 17 *Pythium* strains tentatively identified as *P. debaryanum*.

Because these tests were generally done under aseptic conditions, they indicate only potential pathogenicity of the fungi. It should be understood that the same degree of pathogenicity may not be exhibited in seedbeds. Hartley (4) has shown that the presence of saprophytes may significantly reduce the mortality produced by *Pythium* inoculum. High moisture, moderate pH (2), and carbon sources that stimulate mycelial growth of *Pythium* in the substrate may favor development of the disease in tests such as these; but the effect of these factors, and interaction of field conditions with them, may vary greatly in seedbeds.

One purpose of this study was to find host species suitable as bait for studies of the inoculum potential of *Pythium* in soils. West India gherkin and a few other species near the top of the susceptible groups might be used for this purpose. On the other hand, the intermediate species are the most suitable for measuring the pathogenicity potential of *Pythium* isolates in standardized tests.

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PHYSICAL BARRIERS ASSOCIATED WITH RESISTANCE IN FUSARIUM WILT OF BANANAS

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SUMMARY

The extent of vascular invasion following wound inoculations of Gros Michel banana roots with spore-suspensions of *Fusarium oxysporum* f. *cubense* was limited in upper (young) and extensive in lower (older) roots. Similar differences in the transport of stained spores and carmine lake particles in the transpiration streams of upper and lower roots apparently were the result of physical

changes in vascular structure. Particle and spore passage distances were short in mature protoxylem and early-metaxylem of both young and old roots. Passage distances in functional late metaxylem increased with increasing age because the relatively persistent closing membranes of the perforation plates in these vessels gradually developed large pores and disappeared. Initially, minute pores in these membranes allowed transport only of water soluble dye and colloidally dispersed particles; spores and particles 2 μ or more in size were trapped. Large pores eventually developed in simple transverse and slightly scalariform perforation plates. Vessels up to 323 mm long then allowed passage of large particles and spores throughout their entire length. Such particles were never found to traverse the densely scalariform-pitted vessel endings. Old vessels, because of their great length, commonly allowed passage into the root-rhizome plexus where vessel endings were frequent and entrapment consistently occurred. Passage of particles in old roots was much greater in susceptible Gros Michel than in 2 resistant varieties. Other evidence suggests that physical trapping alone can not provide resistance, but can retard the advance of the pathogen until host-response resistance mechanisms become operative.

INTRODUCTION.—During the course of infection studies of *Fusarium oxysporum* Schl. f. *cubense* (E. F. Smith) Snyder & Hansen, in Gros Michel banana, a considerable variation was found in the extent of root invasion. Preliminary observations suggested that old roots were highly susceptible, whereas young roots were highly resistant to invasion. Furthermore, extensive systemic infection and symptom expression in young potted plants are rare. Rishbeth (7) and Wardlaw (10) also have noted that a considerable degree of resistance to *Fusarium* may be found in the roots and rhizome of the relatively susceptible Gros Michel variety. A study was therefore undertaken to determine the extent and causes for differences in invasion of young and old roots. Since the restriction of infection as described in Gros Michel roots is similar to that described in highly resistant varieties (7, 10), it was hoped that such differences in Gros Michel roots might also suggest possible resistance mechanisms operating in these resistant varieties.

Since this study is intimately related to the anatomy of the banana plant, particularly the xylem of the root, it is necessary to review some of the basic information and concepts associated with these aspects. Although there has been no recent work on the structure of the shoot system of the banana, Simmonds (9) has summarized the earlier work. Pertinent is the fact that the vegetative shoot system of the banana plant consists of a massive, branching rhizome, buds of which produce the aerial portions of the plant. Prior to the period of floral initiation, the entire aerial shoot system (pseudostem) consists essentially of leaves, the overlapping bases of which form a compact cylinder. As the inflorescence develops, there is an elongation of the axis which forces the flower bud through the center of the pseudostem. Roots arise, usually in axial groups of 4, in an acropetal sequence on the corm-like rhizome. At any given time, the uppermost roots are most likely to be the youngest roots; the lowermost roots, the oldest.

In a detailed study of the anatomy of the banana root, Riopel (6) has described the stele of the root as: ". . . a fibrous vascular cylinder which lacks a pith and has, in addition to the common polyarchous radial arrangement of phloem and xylem strands, numerous large vessels and phloem strands in the central portion of the stele." Riopel further considered the 1st formed (outermost) xylem elements to be protoxylem, the remainder of the tracheary elements in the peripheral strand to be early metaxylem, and the inner isolated strands of tracheary elements to be late metaxylem. This usage will be followed in the present paper.

As a result of experiments with carbon particle suspensions, Riopel concluded that the protoxylem elements should probably be interpreted as tracheids —imperforate conducting cells of the xylem (5)— whereas the tracheary elements of the metaxylem are ". . . truly vessels of the most evolved order . . ." A vessel is composed of a superposed series of perforate cells, "vessel members." Terminal members are perforate at 1 end and imperforate at the other (5). Cheadle (3) reported that the later-matured metaxylem as well as the early-matured metaxylem of the roots possesses vessels, as does the inflorescence axis. Vessels were found nowhere else in the shoot system, *i.e.*, rhizome or pseudostem. The ontogeny of the perforation plate is known for relatively few plants (4, 5); but, in general, the loss of the closing membrane has been described as a preliminary swelling of the primary wall material followed by breakdown or dissolution, probably by the protoplast. The existence of small pores in the closing membranes of conifer tracheids has long been recognized (1) and more recently confirmed (2). The presence of pores has not been observed, however, in the pit-closing membranes of tracheids of angiosperms.

MATERIALS AND METHODS.—All plants used in greenhouse studies were derived from "button-type" (dormant bud) rhizome pieces planted in 9-in. clay pots containing a sand and loam soil mixture. They were grown for various periods of time, as specified below, at the Norwood laboratory. Field experiments were conducted on 8-month-old plants at La Lima, Honduras.

Inoculations were made by washing soil from root bases with a jet of water and introducing a few drops of microconidial suspensions (10,000/ml) into root steles approximately 10 cm from the rhizome with a No. 22 hypodermic needle; or by immersing the root system in water, washing the roots by rapid shaking, and similarly puncturing roots under the microconid-

ial suspension. Invasion after 6-36 days of incubation, as specified, was determined by cutting roots longitudinally and measuring the distance of the furthermost vascular discoloration (including discontinuous flecking), from the point of inoculation to the rhizome. For comparative purposes, infected areas were also classed as having terminated: 1) in the root, external to the rhizome; 2) in the root, within the cortical region of the rhizome; or 3) in the root-rhizome vascular plexus.

Stained microspores were obtained by suspending spores from potato-dextrose agar slants in water, filtering through 2-crossed-layers of lens paper to remove debris, adding 5% aniline blue (water soluble), and heating gently. When spores had become intensely stained, they were washed in water by repeated centrifugation. The size of these spores ranged from 2-3 x 3-8μ.

Suspensions of carmine (aluminum lake) particles were often used to confirm results with stained spores since these could be more readily detected and were more readily available in quantity. These suspensions were prepared by adding approximately 3 g of powder to 1 liter of water and agitating vigorously. The suspension was allowed to settle for approximately 5 minutes and was decanted to eliminate very large aggregates. The supernatant was allowed to stand for an additional 2 hours after which the liquid was decanted and the settled particles were resuspended in fresh water. Such a procedure removed most large particles which might cause vascular stoppage and most colloidal size particles which might confuse determinations of passage of spore-size particles. Particle passage and entrapment were determined in water-mounted, free-hand sections of tissues under a binocular microscope. Data were based only on carmine particles 2-8 μ in size.

Functional maturity of protoxylem and metaxylem was determined by the uptake of aniline blue solutions and carmine lake suspensions into roots cut at various distances from their apices. Gros Michel plants for this purpose were grown for 6 months in a friable soil-sand-peat moss (1:1:1) mixture in a bed 12 x 5 x 10 in. deep. Roots were excavated, rinsed, and placed into the test solution immediately after removing 10 or 20 cm of the root tips. After 15 minutes of transpirational uptake, roots were recut under water 20 cm above the initial point of treatment. If no vascular coloration (red or blue) was visible, the remaining portions of the roots were again transferred to the test medium. If particles, or dye, or both were present, no treatment was given; but additional 20 cm segments were removed until no coloration was observed. This treatment procedure was repeated for the entire length of the root to the rhizome.

Transport of aniline blue in the vascular system was determined by microscopic examination of fresh, free-hand cross sections of roots. Transport of spore-size (2-8 μ) carmine particles was determined by microscopic examination of fresh, longitudinal sections of successive root segments. Particle passage distances and causes for partial or complete trapping of particles were noted.

RESULTS.—Three Gros Michel plants, approximately 1-year-old with well-developed rhizomes, were repotted in 12-in. pots and held in the greenhouse for 36 days to obtain vigorous growth of old and new roots. Root bases were washed free of soil by a water jet, punctured, and allowed to take up 3 drops of microspore suspension. After 12 days of incubation, the rhizomes were dissected by vertical-radial plane sections to expose the plexus of each root-rhizome juncture and a longitudinal section of the root (Fig. 1). The peripheral region of the rhizome stele could be subdivided vertically by a color gradient into opalescent (upper), white (intermediate), and yellow (lower) regions. When data were compiled according to the origin of roots, distinct differences in vascular invasion were noted (Table 1). Infections in upper roots usually terminated well outside the rhizome, those in intermediate roots were more extensive but terminated short of the root-rhizome plexus, and those in lower roots generally made successful penetrations to the plexus. There appeared to be, then, a direct correlation between the site of origin of roots and relative susceptibility to vascular invasion by *Fusarium*.

An attempt was made to determine whether differences in resistance were associated with differences in the capacity of these vascular tissues to transport spores. Plants similar to those above were tested with suspensions of carmine or stained spores. Roots were washed free of soil and severed or punctured under a

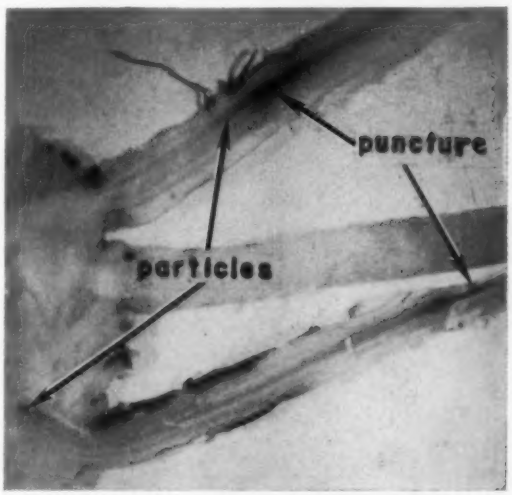

Fig. 1. Limited carmine particle distribution in the xylem of a young root (top) and extensive carmine distribution to the rhizome plexus in an old root (bottom) of a Gros Michel banana plant. Roots were treated for 15 minutes with a water suspension introduced through hypodermic punctures. Distribution was determined in longitudinal sections of treated roots including longitudinal-radial sections of adjoining rhizome tissue.

TABLE 1. Extent of vascular discoloration 12 days after inoculation of Gros Michel banana roots of different rhizome origins (ages) with *Fusarium oxysporum* f. *cubense*

Root origin	% of xylem discolorations terminating in: Root stele outside rhizome	Root stele in rhizome cortex	Root rhizome stelar plexus	No. of roots
Upper rhizome (opalescent)	95	5	0	39
Midrhizome (white)	20	80	0	10
Lower rhizome (yellow)	0	5	95	19

large volume of suspension. After 15 minutes to 2 hours of uptake, under conditions favorable to transpiration, rhizomes and roots were dissected and the extent of vascular coloration was noted. In general, the same pattern of distribution was found for particles and spores as was found following inoculation, *i.e.*, limited in upper roots, extending to the cortical region of the rhizome in intermediate roots, and to the root-rhizome plexus in lower roots (Table 2).

To determine whether this difference in capacity of roots to transport spores or particles could be associated with vascular maturation, the tips of long roots (150-220 cm) were cut off at various distances from their apices. The cut ends were immersed in carmine suspensions with aniline blue for 15 minutes to allow uptake in the transpiration stream. Roots were repeatedly cut and treated at 20- or 40-cm intervals until continuous particle passage to the rhizome occurred. Segments were examined under the microscope, in order of their severing, to determine the extent of dye and particle (2-8 μ) transport from the various treatment points. In late metaxylem, transport of carmine first occurred at approximately 60 cm from the root tip (Fig. 2); the extent of passage, however, was limited. Transport of aniline blue from this point throughout the plant indicated that these vessels were functional in water transport. Failure of particles to move was therefore dependent upon physical obstructions. At greater distances from the root apex, the extent of carmine passage increased. Average carmine transport in the outer large vessels of the late metaxylem appeared to approach a maximum at 120 cm from the apex. Transport in the inner vessels of the late metaxylem appeared to extend somewhat farther. Since these vessels were severed at random distances from their ends, the average distance of particle uptake would be equal to half their average length. Thus, an average central vessel length of 200 mm, or more, was indicated.

TABLE 2. Passage of stained spores and carmine lake particles in the xylem of Gros Michel roots of different rhizome origins (ages) during 15 minutes to 2 hours

Root origin	% of particle passages in root xylem terminating in: Root stele outside rhizome	Root stele in rhizome cortex	Root rhizome stelar plexus	No. of roots
Upper rhizome (opalescent)	93	7	0	15
Midrhizome (white)	16	61	23	13
Lower rhizome (yellow)	0	20	80	46

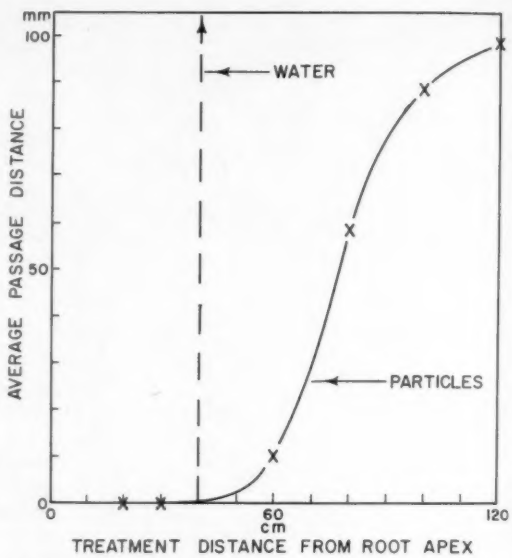

Fig. 2. Relative capacity of late metaxylem elements of increasing maturity to transport water or particles. Gros Michel roots were cut under aniline blue solutions or carmine particle suspensions at various distances from their apices to expose vascular tissues of different degrees of maturity. Distribution of dye or particles after 15 minutes was used to indicate the functional capacity of late metaxylem elements to transport water and *Fusarium* spores, respectively.

During the preceding histological examinations, it became apparent that a screening out of particles and spores occurred at the end-walls of immature vessel members. The end walls were of 2 types; simple and transverse, or somewhat oblique and scalariform. Trapping of particles also occurred at vessel endings which were strongly oblique with dense scalariform pitting. Although this is not intended as an ontogenetic study of the tracheary elements, certain observations were made which are pertinent to the interpretation of particle and spore passage. During the early period of functional maturity (water transport), all closing membranes appeared to remain intact and to prevent passage of spore-size particles (Fig. 3, 4, 5). With increased age, however, transverse and slightly oblique end-walls of vessel membranes developed pores of various sizes which permitted only partial trapping of particles (Fig. 6, 7). These pores appeared to occur

randomly, but with increasing frequency with increasing distance from the root apex. Eventually, the closing membranes of the perforation plates disappeared completely. Particles then passed freely for long distances (as far as 323 mm) to the vessel ending. At no time were large pores found in vessel endings; no spores or spore-size particles were found to traverse them (Fig. 8, 9).

On the other hand, very small pores appeared to be present in all of these membranes at the earliest stages of development, even in membranes of strongly oblique vessel endings. These pores were seen as blank spaces on membranes otherwise coated with fine carmine particles (Fig. 10). That such minute pores do occur in all closing membranes is suggested by the apparent free passage of colloidal-size carmine particles in all elements in early stages of maturity. These particles were found to move throughout the rhizome and into the pseudostem, where only "imperforate" tracheids are presumed to occur (Cheadle, 3).

It was apparent that such end walls could retard the passage of *Fusarium* spores through xylem elements, especially in young roots. That these could effectively prevent systemic invasion, however, was questionable. To clarify this point, it was necessary to estimate theoretical rates of mycelial growth and spore passage assuming no impedence except the physical barriers presented by end-walls. These could then be compared with the actual rate of vascular invasion.

Three series of experiments were designed for this purpose. First, linear growth of mycelium was determined on water, potato-dextrose, and malt agars at 25°C. In all cases a rate of approximately 5 mm/day was found.

In a 2nd set of experiments, the minimum pore size required for passive distribution was determined by filtering microspore suspensions under a slight pressure deficit through cellulose nitrate-acetate discs (Millipore Filters) having porosities of $1.2 \pm 0.3\mu$, $3.0 \pm 0.9\mu$, and $5.0 \pm 1.2\mu$. Spores passed through 3 and 5μ pore sizes, but none passed through the 1.2μ filter. In addition, the time required for growth of mycelium through pores, as well as the time required for subsequent resporulation, was estimated by placing autoclaved filter discs of 1.2 ± 0.3, 0.8 ± 0.05, and $0.45 \pm 0.2\mu$ pore-sizes on water agar in petri dishes. A drop of spore suspension was placed in the center of the upper surface of each disc. Mycelium penetrated discs of all pore sizes in approximately 24 hours. It is possible that this penetration resulted from digestion of the cellulosic filter. The cellulose of primary cell walls is probably less dense than that in the filter; therefore, it is reasonable to assume that these could also be digested. Resporulation on the water agar beneath these filters occurred 3-4 days after initial seeding.

In the 3rd experiment, the rate of *Fusarium* invasion of stelar tissues was determined by inoculating upper roots of Gros Michel plants by means of hypodermic punctures. Vascular discoloration, again used as a measure of invasion, was determined 6, 12, and 36 days later. Young roots were chosen since initial spore passage in such roots would be limited; for this reason, factors other than physical could best be detected there. Vascular discoloration was first evident 4 days after inoculation. First recordings were made at 6 days to allow for differences in rate of discoloration. The average length of discolored xylem at this time (12 mm) was comparable to the extent of vascular coloration resulting from passive carmine particle passage (14 mm). Subsequent measurements indicated a very slow rate of advance (0.2 mm/day).

Theoretical rates of distribution by linear growth and successive spore passages were compared to the actual rate of invasion as indicated by vascular discoloration (Fig. 11). The starting point for each graph line was located 14 mm from the point of inoculation, since this was the average distance of carmine and stained spore passage in the young roots. Subsequently, physical barriers were assumed to occur at an average interval of 28 mm since the average particle passage distance in randomly severed vessels (14 mm) would be half that of intact vessels in these young roots. The line for theoretical advance by means of mycelial growth was based upon a linear growth rate of 5 mm/day and 1 day to penetrate physical barriers. The line for theoretical advance by means of repeated spore passages was based on 4 days required for penetration of physical barriers and resporulation followed by 28 mm of immediate (minutes) free passage to the next barrier. The lag period of 4-6 days required for development of vascular discolorations was considered insignificant since this represented only about 1 mm of extension for any period of incubation after 4-6 days. The theoretical rates for free mycelial growth and repeated spore passages were 22 and 35 times the actual rate of discoloration.

Comparisons of carmine particle transport were made between 8-12-month-old greenhouse- and field-grown plants of the *Fusarium*-susceptible Gros Michel and the highly resistant Lacatan and Valery varieties. Preliminary data for carmine passage in lower roots, in which good transport might be expected, indicate that transport rarely extended into the rhizome in these resistant varieties (Table 3). The average passage distance in the 2 resistant varieties was much less than that in the susceptible Gros Michel, especially in the large field-grown plants.

Discussion.—The means by which field infection of roots and rhizome occur are not well understood. Wardlaw (10) and Sequeira, *et al.* (8) have reported that vascular wounds in roots enhance infection. Direct penetration appears to be limited to roots which are in poor condition (6). Furthermore, microspores are produced abundantly within vascular elements (personal communication from Dr. O. T. Page). Presumably, spores carried in the transpiration stream

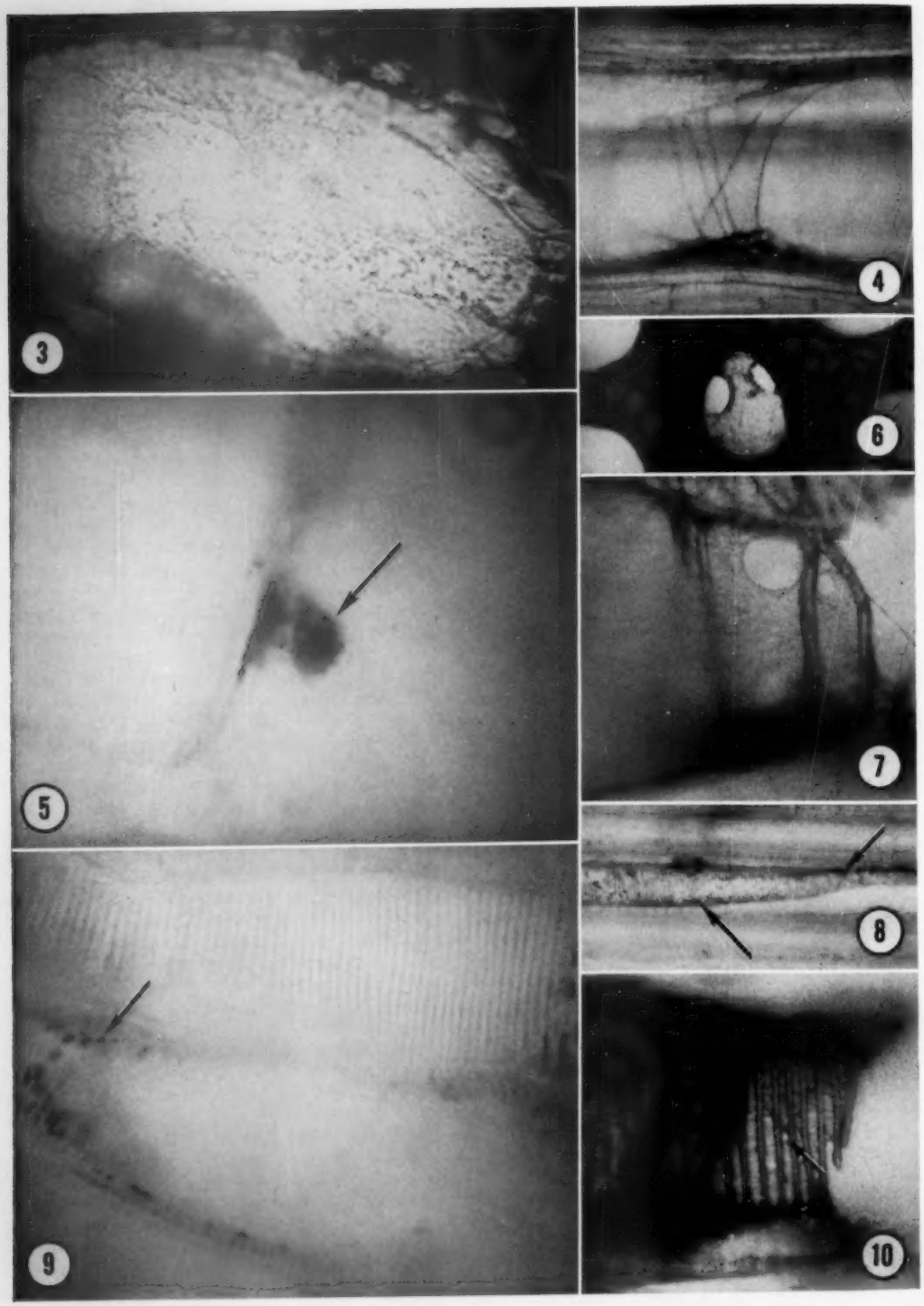
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Fig. 3-10. Portions of functional late metaxylem elements of Gros Michel banana roots. **3)** An intact membrane of a simple transverse perforation plate (× 400). **4)** An intact membrane of an oblique perforation plate (× 390). **5)** Stained microspores trapped by an intact membrane of a simple transverse perforation plate in a young root (× 370). **6, 7)** Pores (5-35μ) in membranes of transverse (× 92) and oblique (× 370) perforation plates. **8)** Carmine particles (× 77). **9)** Stained spores (× 350) trapped at scalariform-pitted vessel endings. **10)** A small (3μ) pore in a pit membrane of a vessel ending (× 450).

could be effective in distributing the pathogen whether initial infection occurred by spores introduced through wounds or by direct mycelial penetration. Inoculations in these experiments were made by introducing microspores into punctured or severed roots, since this apparently eliminated many complicating external factors and allowed better study of internal resistance mechanisms.

In the experiments with roots of Gros Michel plants, the extent of vascular invasion as indicated by vascular discoloration was closely correlated with the extent of spore and particle passage in the transpiration stream. *Fusarium* invasion and particle passage in young vascular tissues of roots occurred only in protoxylem and early metaxylem. These tissues are composed of imperforate tracheids in protoxylem (6) and of short vessels in early metaxylem. No spore-size carmine particles were observed to traverse the vessel endings. The late metaxylem in these younger roots, although sometimes functionally mature as evidenced by the transport of aniline blue, was not structurally mature and did not permit the passage of particles because of the intact closing membranes of the perforation plates in the end-walls of the vessel members. The short length of the protoxylem tracheids and the early metaxylem vessels, and the structural immaturity of the late metaxylem, therefore, prevented extensive particle passage and extensive invasion by the pathogen in young root tissues.

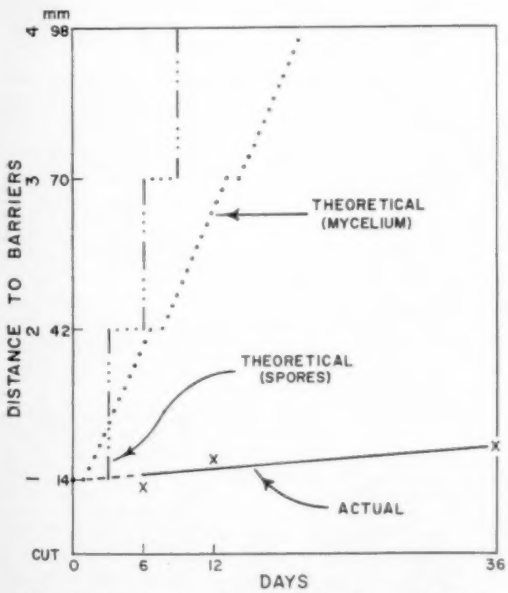

Fig. 11. Evaluation of physical barriers in restricting distribution of *Fusarium* in root xylem vessels by a comparison of actual rate of vascular discoloration with theoretical rates of mycelial growth or spore passage. See text.

TABLE 3. Average maximum vascular passage of stained microspores or carmine lake particles in lower roots of greenhouse- or field-grown susceptible Gros Michel and resistant Lacatan and Valery varieties

Passage	Varieties		
	Gros Michel	Lacatan	Valery
Avg. distance in mm			
Greenhouse	98[a]	67	40
Field	380	26	21
% to plexus			
Greenhouse	85	0	7
Field	100	0	0
No. of roots			
Greenhouse	51	12	27
Field	6	6	6

[a] This distance (98 mm) probably was limited by the average length of severed roots which was only 105 mm.

In older vascular tissues of roots, extensive passage and infection were found in late metaxylem. Each long late-metaxylem vessel is composed of numerous inner members having transverse simple or slightly oblique scalariform perforation plates and 2 terminal members the outer ends of which have long, tapered, densely-pitted end-walls which form the vessel endings. Although the closing membranes of the perforation plates persisted after vessels became functional with respect to water transport, large perforations gradually developed. Eventually, those closing membranes disappeared entirely. Free particle uptake and passage throughout the length of several vessels then occurred. In no case, however, did spores or spore-size particles traverse the tapered, scalariform-pitted vessel endings.

Since these observations have been made from fresh, free-hand sections, cytological changes which may accompany loss of closing membranes were not determined. It would appear that, since the ontogenetic sequence described here is somewhat different from that described by other workers (5), a strictly ontogenetic study, using methods of the plant histologist, may be most productive.

Limited invasion in the young root tissues described above could then be accounted for by screening out of spores from the transpiration stream by vessel end-walls of short protoxylem tracheids and early metaxylem vessels, or by closing membranes of perforation

plates in late metaxylem. More extensive invasion and the common "flecking" symptom of vascular discoloration could be accounted for by partial perforation of late metaxylem elements which resulted in only partial trapping at successive membranes. Finally, invasion to the plexus in old root tissues was dependent upon free passage of spores throughout the length of late metaxylem vessels to the terminal end-walls. Since vessel endings occur randomly within the root, not all vessels in old roots allowed passage to the root-rhizome plexus. The closer to the rhizome a root was severed, however, the greater was the number of vessels which allowed passage to the rhizome.

Confinement of invasion to a root could mean merely the functional loss of that root or even only a portion of the root since the remainder often appeared to be vigorous and functional after long periods of incubation. Root losses, from numerous causes, occur normally. New roots are continually produced. Limited root infections, unless numerous, would therefore seem to be of little significance to the plant as a whole.

Invasion to the rhizome plexus would be of greater significance since infection here would have systemic potential. However, physical barriers at the root-rhizome plexus also were pronounced. In the root-rhizome vascular plexus, there is, in a very short distance, a transition to very short vessels, until in many instances there is only 1 vessel member in each vessel. These single elements are, by definition, tracheids. Spore-size particles, as well as vascular infection, did not traverse these plexal barriers within the time and environmental limits of these experiments.

Rishbeth (7), after studying infection in resistant and susceptible varieties in the field, reported that only a small proportion of root infections penetrated to the rhizome stele in any variety, but that infection was more restricted in the resistant varieties. Wardlaw (10) also reported a slower rate of root penetration and fewer rhizome infections in the highly resistant Lacatan than in the susceptible Gros Michel. These reports suggest that, in both varieties, invasion can be restricted and that a quantitative difference exists between varieties which exhibit practical field resistance or field susceptibility.

Particle passage in greenhouse- and field-grown resistant and susceptible varieties showed such quantitative differences in the ultimate passage distances in mature metaxylem. Anatomical differences, therefore, could account for these quantitative differences in resistance. If such were the case, wounds close to root bases of even these resistant varieties could permit plexal infection. This assumption is supported by occasional passage of particles to the plexus and actual plexal infections which may occasionally be found in resistant varieties (7).

Once penetration to the plexus has taken place, however, additional physical barriers are encountered within the plexus, similar to those in Gros Michel. These appear to restrict further the distribution of the pathogen into the rhizome. Such barriers in both resistant and susceptible varieties are currently being studied in relation to systemic distribution.

That physical barriers are of considerable importance in *Fusarium* wilt is indicated by the rapid, initial penetration to the 1st physical barrier. On the other hand, such barriers, including vessel endings, were ineffective in stopping the passage of bacterial-size colloidal particles. These particles were found to pass through roots, plexus, and rhizome into the pseudostem in a very short time in all 3 varieties. It seems significant that complete invasion and wilt of large plants may occur within a few days after inoculation with *Pseudomonas solanacearum* E. F. S. and that no varietal resistance to this bacterial wilt is known. In the case of bacterial wilt, the ineffectiveness of physical barriers appears to be correlated with rapid wilting and general absence of resistance. In the case of *Fusarium* wilt, effective functioning of such barriers appears to be correlated with various orders of resistance.

An attempt was made to evaluate physical barriers as a sole means of resistance to *Fusarium*. Comparisons were made between actual rate of vascular invasion, as indicated by discoloration, and theoretical rates of unobstructed mycelial extension and successive spore passages from barrier to barrier. Both theoretical rates included delays at successive barriers. These represented the time required for the fungus to grow through small pores that exist even in young, intact membranes. The rate of actual advance was found to be only a fraction of that estimated for mycelial growth or successive spore passages. These results indicate that physical barriers alone are inadequate to account for the observed resistance to invasion.

Wardlaw (10) has described cortical and vascular growth responses in susceptible Gros Michel and resistant Cavendish banana varieties which may restrict invasion by *Fusarium*. Such host response mechanisms would require time to become operative; and would, on this basis, seem inadequate, *per se*, to contain an infection.

It is proposed, therefore, that both physical barriers and physiological responses are essential to resistance; and that, under normal environmental conditions, temporary trapping of the pathogen in these physical barriers provides the time necessary for pathogen-induced resistance mechanisms to become operative.

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APHID TRANSMISSION OF BARLEY YELLOW DWARF VIRUS IN WISCONSIN

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SUMMARY

According to studies on acquisition and inoculation feeding as well as on the retention of the virus by its aphid vector, barley yellow dwarf virus (BYDV) is a typical persistent virus. Vector specificity, retention of the virus after molting, and a possibility of mechanical transmission of the virus into a vector were considered as additional proof of its persistent nature.

Rhopalosiphum poae is reported as a vector of BYDV for the 1st time but this species appeared to be of little importance in the epidemiology of the disease. Three other grass aphid species tested were unable to transmit the virus. Root inoculation of small grains was not considered of epidemiological consequence.

In the field, quantitative relations between number of vectors and number of diseased plants existed. With number of vectors restricted in 1957 and 1958, yellow dwarf appeared to be endemic in Wisconsin, while exceptionally high numbers of *Toxoptera graminum* resulted in an epidemic of the disease in 1959. Indications were that less than 1% of the grain aphid population carried the virus. The role of native grasses in virus acquisition has not been clearly defined, although it is considered secondary. Frequent insecticidal spray caused a marked reduction of virus incidence in the field.

Introduction.—One purpose of studying insect transmission of virus diseases is to establish various factors which govern the distribution and frequency of the disease as it invades populations of host plants. After the basic work of Oswald and Houston (12) on aphid transmission of barley yellow dwarf virus (BYDV), the existence of vector-specific strains was pointed out by several workers (3, 17, 22). Recently, Rochow (18) and Toko and Bruehl (22) have given detailed accounts of the inter-relationships between 2 common aphid vectors and strains of BYDV. Stetson, *et al.* (20) and Orlob and Medler (14) have studied development of aphid populations in relation to BYDV in 2 widely separated parts of the United States. Although these studies have provided considerable information on how this virus is transmitted, much remains to be learned about the epidemiology of the disease.

In this report, various experiments and observations will be reported on the transmission of BYDV in the greenhouse and in the field under Wisconsin conditions.

Results.—*Barley yellow dwarf—a persistent virus.*—A study of virus transmission in the greenhouse was made to study the factors influencing perpetuation of the disease. To determine acquisition and latent periods, aphids of the *Rhopalosiphum fitchii* (Sand.)—*R. padi* (L.) complex (which have been called the apple grain aphid in the United States) were fed for various times (1-60 hours) on barley infected with BYDV isolated from several naturally infected grain plants (Wisconsin strain of BYDV). These aphids were transferred in groups of 4-6 to individual barley plants (C.I. 666) for various inoculation feeding periods. The results (Table 1) indicated that the apple grain aphid could acquire the virus in as short a time as 1 hour. Efficiency of transmission, however, increased with more time on the source plants, as more aphids apparently became viruliferous after they fed for 19 hours or longer on infected plants. In other experiments, *R. fitchii* acquired the virus in 9 hours,

TABLE 1. Summary of acquisition and latent periods of *R. fitchii* in transmitting BYDV during 1957 and 1958; 4-6 aphids were fed on individual plants—Numerator indicates number of infected plants and denominator total number of plants tested—Hyphen indicates no experiments performed

Acquisition feeding (hours)	Inoculation feeding time (hours)									
	1	2	3	4	6	10	12	16	24	48
1	0/10	0/12	–	–	0/10	–	–	0/12	–	1/10
2	0/5	0/10	0/12	–	0/5	–	–	0/5	–	0/12
3	–	0/8	–	–	–	0/8	–	–	0/6	0/8
4	–	1/10	–	0/10	–	–	0/5	–	–	1/12
5	0/5	0/6	–	0/6	–	–	–	–	–	1/12
6	–	0/10	–	–	–	–	0/4	–	–	0/10
7	–	0/5	–	–	–	–	0/5	0/5	–	1/10
9	1/10	–	–	–	–	–	0/5	–	–	1/10
19	–	–	1/5	–	1/5	–	–	–	–	2/5
24	1/5	–	1/8	–	–	–	2/8	–	–	8/10
60	–	2/5	–	–	–	–	–	–	–	4/5

but efficiency increased with longer feedings. Although this species could accomplish the whole transmission cycle in as short a time as 6 hours, usually a much longer period (24-48 hours) was necessary before transmission occurred regularly. These results indicate that under most conditions a latent period occurred in the transmission of BYDV, which, however, like in other persistent viruses, seemed to be of variable length.

R. fitchii and the rose grass aphid, *Macrosiphum dirhodum* (Wlk.), were reared for at least 1 week on infected barley plants. After fasting 1 hour, 5-10 aphids were placed on each healthy barley test plant for different periods of time. *R. fitchii* transmitted BYDV in a few cases if fed for 30 minutes on test plants, but infection increased to 26% when aphids fed for 1 hour. *M. dirhodum* gave no transmission in 30 minutes, but infected 4.7% of the healthy plants during a 1-hour feeding period (Table 2).

Differences in efficiency between these 2 species also were noticeable when individual viruliferous aphids were allowed to feed for 24 hours on individual test plants, then transferred to a 2nd plant for 24 hours, and so on. In both species, individual aphids transmitted the virus as long as they lived, although not every plant in a series was infected. *M. dirhodum* infected successive plants less regularly than did *R. fitchii*.

Studies on the retention of BYDV in 2 vector species confirmed earlier work (18, 22) which described the virus as persistent. Viruliferous *R. fitchii* and *M. dirhodum* were kept in small vials at 2-4°C for 6, 8, 10, and 12 days, before transfer to healthy seedlings. All test plants were infected by both species, indicating that the virus was retained during fasting periods under cold conditions. Comparable results were obtained when viruliferous corn leaf aphids, *Rhopalosiphum maidis* (Fitch), were fed on maturing corn which is immune to some, but not all (23), strains of BYDV. Aphids transmitted the virus to healthy plants after being on corn for 9 days.

Alatoid nymphs (*R. fitchii*) approaching the molting stage were collected from virus-infected plants and placed in petri dishes. After molting into alate forms, the aphids were transferred to test plants. Ten aphids were placed on each of 6 plants, all of which became infected. Retention of infectivity following ecdysis is considered by several workers (8, 10, 21) as evidence that the virus is not carried mechanically on the stylets but is present in the body of the aphid.

Mechanical transmission of BYDV from vector to vector.—It has been known for some time that certain aphid-borne persistent viruses can be transmitted mechanically to their vectors (8). Limited experiments were made in an attempt to introduce BYDV directly into *M. dirhodum*.

Aphid macerate and haemolymph were introduced into virus-free aphids by means of Minuten insect pins. Such aphids were then fed on healthy grain plants to determine whether or not they were viruliferous. Two hundred and fifteen aphids into which haemolymph had been introduced gave only 1 infected plant and 155 subjected to aphid macerates infected only 2 plants. In parallel injections of the 6-spotted leafhoppers [*Macrosteles fascifrons* (Stal.)] with aster yellows virus, 1 out of 40 leafhoppers was made viruliferous.

With this method, BYDV was not as readily transmitted from vector to vector as was aster yellows virus. Mueller, *et al.* (11) have recently reported successful injections of BYDV into 2 species of aphids using micro-injection needles.

Vector species.—In the present study various grass and cereal aphids occurring in the Madison area, not reported by Oswald and Houston (12) as vectors of BYDV, were tested for their ability to transmit the virus.

The aphid species were collected in the field and confined to infected barley plants in the greenhouse. With the exception of *Hyalopterous arundinis* (Fabr.) which is monophagous on *Phragmites communis* Trin., all grass aphids tended to multiply normally on barley after an initial period of adaptation. Adult apterae from well-established colonies were then placed on test plants using the method described by Oswald and Houston (12).

In these tests *Rhopalosiphum* (*Rhopalomytus*) *poae* (Gill.), the bluegrass aphid, transmitted BYDV to

TABLE 2. Inoculation period required for 2 aphid species to transmit BYDV—Acquisition period at least 1 week—Numerator indicates number of infected test plants—Denominator indicates total number of plants tested in 1957 and 1958

Aphid species	Inoculation feeding periods			
	15 min	30 min	45 min	60 min
R. fitchii	0/114	5/145	16/149	34/131
M. dirhodum	0/121	0/141	0/119	6/128

87% of the barley test plants (in comparison, *R. fitchii* infected 88%, *M. dirhodum* 40%, and *Toxoptera graminum* (Rond.), the greenbug, 44%). Insofar as we are aware, this is the 1st report of the bluegrass aphid as a vector of BYDV. No transmission was obtained with the following species: *Sipha agropyronensis* (Gill.), the quackgrass aphid; *Hysteroneura setariae* (Thom.), the rusty plum aphid; and *Myzus persicae* (Sulz.), the green peach aphid. The primary food plants of the quackgrass and rusty plum aphids are grasses which are immune to BYDV, whereas *M. persicae* has a wide host range but usually is not found on grasses in nature.

Although *R. poae* is a little-known aphid, it is fairly common during the fall in its preferred breeding areas. It was not found during spring or summer and its life cycle is not entirely known (15). Probably the aphid does not contribute to the spread of BYDV in spring grains, although it might serve to spread the virus in bluegrass.

Several root aphid species (in the tribes Pemphigini and Fordini) were unable to transmit the virus after feeding on infected roots. Although apple grain aphids became viruliferous after feeding on roots of infected plants, test plants failed to develop symptoms when the inoculation feeding was on the roots. Since the root aphids would not feed on tops, it is not known whether they were able to serve as vectors.

The English grain aphid, *Macrosiphum granarium* (Wlk.), acquired BYDV from dodder (*Cuscuta campestris*) established on an infected barley plant. Direct transmission by the dodder was not demonstrated.

Seasonal transmission of the virus.—The aphids colonizing summer hosts may come as migrants from the south or from Wisconsin winter hosts. In this colonization, they may introduce and spread the virus. Generally, the extent of crop infection will increase in proportion to aphid populations, but there are exceptions (2). Some work along this line has been done by Stetson, *et al.* (20), but they did not correlate aphid populations with development of disease in the field. In the present study, the relation between airborne populations of vectors and the occurrence of yellow dwarf throughout the growing period was investigated.

Plots (40 m^2) were planted at about 2 bu/A instead of the usual 2.5-3 with susceptible oat and barley varieties. Yellow pan and wind traps were placed in these plots and the trapping results recorded. The total number of cereal aphids at 5-day intervals was plotted against number of diseased plants in the plot. After approximately 1 month, similar plots were planted near by, traps were transferred to the new planting after emergence of seedlings, and the old plots were plowed under.

In 3 successive growing seasons there was a positive correlation between the number of spring migrants trapped and disease counts in the plot (Fig. 1). Two to 4 weeks elapsed between the highest aphid counts

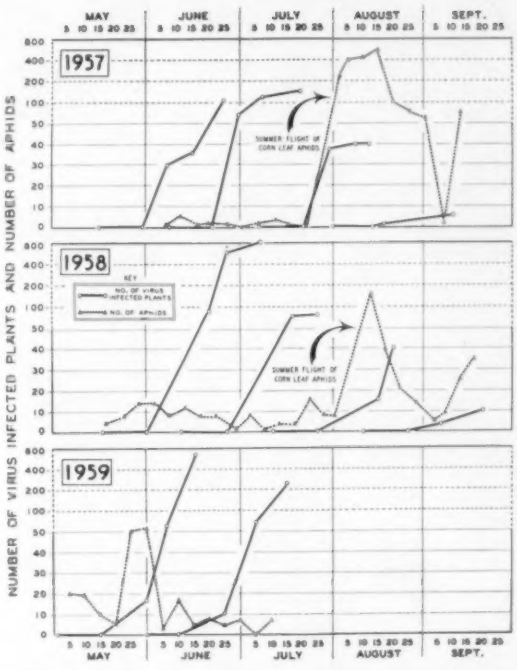

Fig. 1. Aphid numbers in yellow pan and wind traps and incidence of yellow-dwarf-infected plants in grain plots planted at intervals during the growing seasons of 1957, 1958, and 1959.

and the greatest number of diseased plants. The plots planted in May and June had the most aphids, both in traps and on plants, and the maximum number of infected plants. In 1959, counts reached comparable numbers 2-3 weeks earlier than in the previous years and rose to higher levels, due to a high population of *T. graminum*. During midsummer the curves of disease incidence began to drop, although the curves of aphid numbers continued to rise, due to the abundant appearance of *R. maidis* (Fig. 1, summer flight of corn aphids). This phenomenon could be explained by the fact that most of the corn aphids came from corn which is immune to many strains of BYDV and thus served as a "virus filter." With the exception of *T. graminum* in 1959, all other vectors common during May and June failed to increase and disappeared almost completely by early July.

Proportion of vectors which were viruliferous.—Davies and Whitehead (7) collected 1,178 *M. persicae* in the field, made inoculation feedings of 5-20 aphids/plant, and found that only 4 test plants were infected with potato leaf roll, and 2 with virus Y. Stetson, *et al.* (20) reported finding that 28% of apple grain aphids and 40% of English grain aphids carried BYDV.

Alate *M. granarium* and *R. fitchii* that were collected in the field during May and June of 1957 and 1958 were placed on test plants in groups of 1-5 aphids/plant. In 1957, 150 *M. granarium* infected 1 plant

and 60 *R. fitchii* also infected 1 plant; in 1958, 200 *M. granarium* gave 1 infected plant whereas 56 *R. fitchii* gave none. As these experiments were conducted only over a 2-year period, no conclusion can be made on the variation of viruliferous spring migrants from year to year. In the date of planting experiment, however, the number of diseased plants in the plots seemed to be associated with numbers of viruliferous vector species trapped in a given season. In the June planting of 1957, 4 *M. granarium* and 2 *R. fitchii* were trapped (yellow pan) and 145 infected plants were found in the plot, while 545 *R. maidis* were trapped (yellow pan) in the August planting and only 5 infected plants developed. In the May planting of 1958, 23 *M. granarium*, 10 *R. fitchii*, and 2 *R. maidis* were trapped (wind and yellow pan traps) and 805 plants became infected, while in the August planting a catch of 161 *R. maidis* was associated with only 10 infected plants in the plot. Thus, the spring population had a greater proportion of viruliferous individuals than the summer population of aphid vectors.

Host plants of the virus.—The question of how aphids become viruliferous is of primary importance in disease epidemiology. Extensive lists of grass hosts of BYDV have been presented (4, 13, 23). In the present study, the virus was recovered from the following grasses after artificial inoculation: *Poa pratensis* L., *Phleum pratense* L., *Lolium perenne* L., *Festuca rubra* L., *Setaria lutescens* (Weigel) F. T. Hubb., and *Digitaria sanguinalis* (L.) Scop.; all except the last have been reported previously as hosts of BYDV. *D. sanguinalis* was the only grass developing symptoms of the disease, becoming yellow with slight dwarfing. When winter wheat was artificially inoculated in the field during October, the virus was recovered the following May from plants which then showed considerable stunting.

In evaluating the epidemiological role of grasses as virus hosts, observations on the feeding habits of aphid vectors showed that all species feed most frequently on cereals and were quite rarely found on any single perennial grass susceptible to the virus. Thus, the problem of virus acquisition must be visualized as a problem of food preference. Spring migrants may become viruliferous either in areas farther south where over-wintering of aphids on grain favors a higher density of virus inoculum, or by occasional feeding on winter grain or grasses in Wisconsin. As to the 1st possibility, symptoms of BYDV appeared in oat fields on May 23, 1959, 17-21 days after a heavy infestation of greenbugs possibly from areas far to the south (14). Spring grain, which is the preferred host of the vectors, is available when most of the spring migrants appear; the chance of aphids picking up the virus from secondary food plants seems low. Early migrants, however, that arrive throughout April before spring grain is available, may be forced to feed on grasses and thus acquire the virus before they migrate locally to spring grain. After ripening of grains, small and scattered populations of some vector species are maintained on grasses.

TABLE 3. Control of barley yellow dwarf by repeated insecticidal sprays—Plots 6 x 7 ft with alternating rows of susceptible oats and barley—1 plot sprayed 3 times/week with parathion, 1 sprayed once/week with Systox (demeton), and each had an adjacent nonsprayed plot—Plots surrounded by cultivated soil—Infected plants counted on 2 dates each year

Date of reading	Number of infected plants with given treatment							
	Parathion				Systox			
	Sprayed		Nonsprayed		Sprayed		Nonsprayed	
	1958	1959	1958	1959	1958	1959	1958	1959
6/7	1	2	2	15	0	4	1	9
6/27	9	12	29	49	9	15	27	52

Virus control by spraying insecticides.—Yu (24), in an attempt to control "red leaf"—a persistent virus similar to BYDV except in the vector and host range—in northern China, found that differences in disease incidence between plots sprayed with insecticides and untreated plots were not significant. Arny and Medler (unpublished 1956 and 1957 results) obtained a slight increase in yield when 4 sprays of a Systox-Guthion-Phosdrin mixture was applied to oat plots. Caldwell, *et al.* (6) reported a very striking control of the disease in oats in 1959 through the use of an insecticide to control the aphids.

Attempts were made during a 2-year period to investigate the possiblity of reducing BYDV incidence by using insecticides under optimal conditions for aphid infestation. In 1958 and 1959 several plots (6 x 7 ft) consisting of 7 rows each of a susceptible barley and oat variety were thinly planted during late April. These plots were distributed at random in a freshly-cultivated field and strips of bare soil kept around them. After seedling emergence, parathion (1:1,000) was applied 3 times weekly to 1 plot and Systox (1:1,000) once a week to another. Two nonsprayed plots served as checks. The incidence of BYDV was recorded in sprayed and nonsprayed plots.

Introduction of BYDV by incoming vectors as well as secondary spread within the plot was reduced as indicated in Table 3. The insecticides were about equally effective.

DISCUSSION.—Acquisition and latent periods appear to be of biological importance in virus transmission by leafhoppers. With the evidence at hand, this cannot be said for aphid-borne persistent viruses. Many workers agree that these periods are not of definite length for persistent viruses (8, 9, 13).

Toko and Bruehl (22) found an acquisition feeding of 24 hours followed by an inoculation feeding of 3-4 hours necessary for infection. On the other hand, acquisition and inoculation periods of 10 minutes have been reported as sufficient (1). In the present study, acquisition feeding of at least 7-9 hours and inoculation feeding of 1 hour were required for consistent transmission of BYDV.

Results obtained in transmission studies of BYDV compare with those of potato leaf roll—the most thoroughly studied aphid-borne persistent virus. For this virus, several investigators have reported an acquisition and latent period of hours or days, while another group found that the whole transmission process could be accomplished in as short a time as 20 minutes (8, 9).

Some of the epidemiological work on virus transmission was concerned with the relative abundance and importance of several potential vectors under field conditions (2). Previous studies on barley yellow dwarf assumed *M. granarium* and *R. fitchii* were the principal vectors (3, 17, 22). During the 3-year period of research here reported, the vector complex was divided into 2 groups. Endemic vector species (*M. granarium, R. fitchii, R. maidis*) were present in the same proportion throughout the observational period. Because of its abundance in 1959, and its close association with a severe outbreak of BYDV disease, *T. graminum* was considered an epidemic vector.

In accordance with Bruehl, *et al.* (5), it was found that largest numbers of aphids were most likely to cause highest amounts of infection in grain fields. Conditions in Wisconsin, however, are quite different from those in Washington. In Wisconsin, endemic vectors rarely find conditions suitable for the build-up of high populations which are essential for the disease to assume some importance in short-season spring grain. A different epidemiological picture was presented in 1959. The population density of *T. graminum* was high in areas south of Wisconsin, and an extensive warm front allowed great numbers of greenbugs to be moved northward during a 5-day period. Some viruliferous migrants introduced the virus, and a subsequent rapid build-up of aphids resulted in secondary spread within the crop and between different fields (14).

Few data are available on the proportion of wild host plants which serve as a virus reservoir in nature. Schwarz (19) found 69 weed species in the Berlin area infected with several nonpersistent viruses. Quite different conditions can be expected with BYDV. As most of the vector species prefer grain plants as a habitat, they rarely become associated with grasses. Toko and Bruehl (22) suggested that *R. fitchii* and *M. granarium* would not remain long enough on grass either to acquire or transmit the virus, and that relatively few grasses serve as important virus reservoirs. Oswald and Houston (13) and Rademacher and Schwarz (16), however, considered grasses important under certain conditions when aphids move from these hosts to grain. Each assumption may be true under the particular conditions involved. There is need to study more critically the role of grasses in the epidemiology of the disease.

Aphids may be more likely to acquire BYDV in areas (southern and western United States) where virus foci are prevalent because of a year-round association between vector and grain. Few viruliferous aphids disperse to areas where these conditions do not exist. This, together with the fact that a relatively few vectors will feed on grass reservoirs of the virus, makes it necessary that the populations of migrant aphids be extremely high for yellow dwarf to assume serious proportions in Wisconsin.

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RATE OF REPRODUCTION OF MELOIDOGYNE SPP. AS INFLUENCED BY SOIL TEMPERATURE

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SUMMARY

The influence of soil temperature on egg-mass production by 6 populations of *Meloidogyne javanica javanica* and *M. incognita acrita*, 2 populations of *M. hapla*, and 1 each of *M. arenaria arenaria* and *M. arenaria thamesi* was determined. In 35 days, only a few egg masses were produced at 15-16°C. The number of egg masses produced increased rapidly at 20-21°C. Maximum egg-mass production occurred for most species in the range of 25-32°C. A number of egg masses containing viable eggs were produced by populations of *M. incognita acrita* and *M. javanica javanica* at 35°C. At soil temperatures above 30°C, egg-mass production by the 2 *M. hapla* populations studied was significantly less than that by *M. incognita acrita* and *M. javanica javanica*. The population of *M. arenaria thamesi* studied produced egg masses at 35°C, while reproduction of *M. arenaria arenaria* was limited above 32.6°C.

INTRODUCTION.—In the hot interior deserts of the southwestern United States, root-knot nematodes (*Meloidogyne* spp.) have been observed to develop at soil temperatures ranging from 30-35°C. Little detailed work on the ability of the root-knot nematodes to produce viable eggs at high soil temperatures has been reported since Chitwood (2) described the several species of *Meloidogyne*.

Godfrey (4) recorded the production of galls on lettuce roots at 35°C and on tomato roots at 40°C. Data on egg production were not given. Tyler (7) reported that the maximum temperature at which a root-knot nematode would produce eggs was 31.5°C. The species of *Meloidogyne* used by Godfrey and Tyler are not known. A preliminary paper on a portion of this work reported that certain *Meloidogyne* spp. may differ in their ability to produce eggs at high soil temperatures and that some production of viable eggs was possible above 31.5°C (6). Bergeson (1) recently demonstrated that *M. hapla* Chitwood, 1949, was better able to survive low temperatures than *M. incognita acrita* Chitwood, 1949.

This study was undertaken to determine the maximum temperature at which different species of *Meloidogyne* could produce eggs and to study further the differences between species in their rates of reproduction at various temperatures.

METHODS.—All tests were conducted in a steamed, sandy loam soil. Rutgers tomato seedlings were used in most tests; however, *Sesbania exaltata* (Raf.) Cory, a leguminous weed that commonly grows in the desert valleys of southern California, was used in a portion of the tests because of its ability to grow at soil temperatures above 30°C. Seedlings were transplanted into pots and all were grown for several weeks at the same soil temperature. The soil was then adjusted to the proper temperature before introducing the nematodes.

All root-knot nematode populations were maintained on tomato. Larvae obtained from egg masses on tomato roots were used to infest the soil in all cases. A water suspension of larvae was pipetted into probe holes around the base of the plants. The size of the container in which the plants were grown and the number of larvae introduced varied with the several experiments and will be given with the results from each.

Soil temperatures were maintained to ±0.5°C by immersing the pots in water in temperature tanks. Plants were grown for approximately 30-35 days (time for only 1 generation) after the nematodes were introduced into the soil. The roots were carefully washed free of soil and examined for egg production (as indicated by egg masses) by the nematodes.

TABLE 1. *Meloidogyne* populations used in soil temperature tests

No.	*Meloidogyne* sp.	Location in California	Host	Type of population used*
1	*M. javanica javanica*	Arvin	Peach	S
2	*M. javanica javanica*	Nicholaus	Tomato	S
3	*M. javanica javanica*			S
4	*M. javanica javanica*	Fresno	Alfalfa	F
5	*M. javanica javanica*	Mecca	Bermudagrass	S
6	*M. javanica javanica*	Chino	Cowpea	S
7	*M. incognita acrita*	Davis		S
8	*M. incognita acrita*	Santa Ana	Tomato	S
9	*M. incognita acrita*	Loomis	Peach	S
10	*M. incognita acrita*	Yountville		S
11	*M. incognita acrita*	Madera	Grape	S
12	*M. incognita acrita*	Livingston	Grape	S
13	*M. hapla*	Davis		S
14	*M. hapla*	Badger Canyon	*Erodium* sp.	F
15	*M. arenaria arenaria*	Coachella	*Lippia* sp.	S
16	*M. arenaria arenaria*	Lodi	Grape	S

*S = single egg-mass population, F = field population.

In the 1st series of tests, the roots were scored from 0-4, using a modification of the system of Sasser (5). The amount of root galling and reproduction was scored as follows: *0*—no reproduction, or if larvae entered the roots, they did not develop into mature egg-laying females; *1*—extremely light reproduction with only an occasional mature female with egg mass; *2*—light reproduction with mature females and egg masses easily seen; *3*—moderate reproduction with females and egg masses moderately abundant; *4*—heavy reproduction with mature females and egg masses abundant. This system was unsatisfactory for measuring the rate of reproduction, even though emphasis was placed on presence of egg masses.

In all subsequent tests, the number of egg masses on the roots of each plant was counted. In the 2nd series of tests, the number of galls, as well as the number of egg masses, is given. The origin of the several root-knot nematode populations used is shown in Table 1.

RESULTS.—*Series 1.*—In the 1st series of tests the tomato seedlings were planted in soil in 2-gal crocks and the soil in each infested with 5,000 active larvae. Two populations each of *M. hapla* and *M. javanica javanica* (Treub, 1885) Chitwood, 1949, were used in these tests and 1 each of *M. arenaria arenaria* (Neal, 1889) Chitwood, 1949, and *M. incognita acrita*. All results (Fig. 1) are averages of 2 tests, 4 replicates/test, except those from population 14 of *M. hapla*, which are an average of 1 test.

With 1 exception, moderate to abundant galling and egg-mass production occurred for all species at 20°, 25°, and 30°C. Egg-mass production was light for population 14 of *M. hapla* at 20°C. Development of the 2 *M. hapla* populations and *M. arenaria arenaria* at 35°C was limited to the production of a few galls and an occasional egg mass. Production of egg masses by population 7 of *M. incognita acrita* and 4 of *M. javanica javanica* was sharply reduced at 35°C. Only population 6 of *M. javanica javanica* produced numerous egg masses at 35°C. These preliminary tests indicated that egg-mass production by *M. hapla*, and possibly by *M. arenaria arenaria*, was restricted above 30°C to a greater degree than that of the other 2 species. However, critical evaluation required that counts be obtained on number of egg masses.

To determine whether the eggs observed in the egg masses on tomato roots growing at 35°C were viable, the roots in 1 test were used to infest clean soil in which tomato seedlings were planted. The roots had been growing in soil infested with *M. incognita acrita* population 7, *M. javanica javanica* population 4, and *M. hapla* population 13. *M. incognita acrita* and *M. javanica javanica* had produced egg masses on the roots of the tomatoes at 35°C, but *M. hapla* had not. After 5 weeks the roots of tomato seedlings planted in soil infested with roots from the *M. incognita acrita* and *M. javanica javanica* treatments were heavily

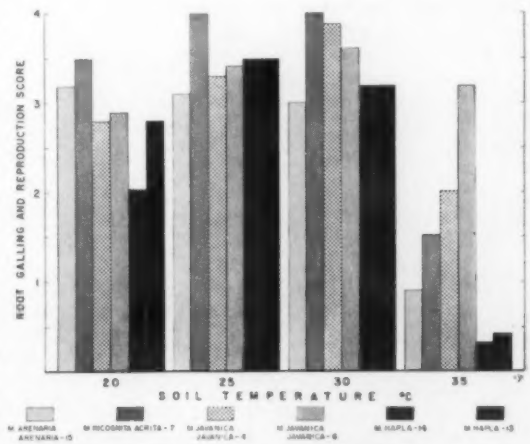

Fig. 1. The influence of soil temperature on the reproduction of *Meloidogyne* spp. on tomato.

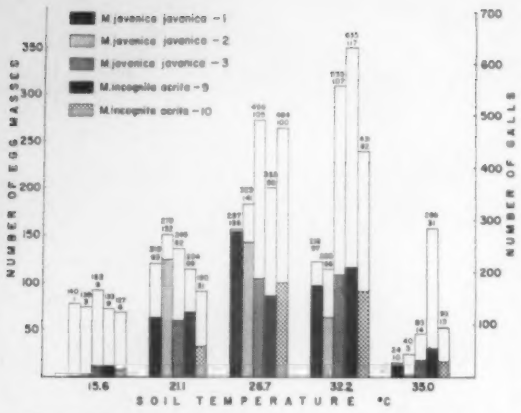

Fig. 2. Influence of soil temperature on root-galling and egg-mass production by *Meloidogyne* spp. on tomato. Bars representing both galls and egg masses have the same base line. Top and bottom numbers above each bar indicate numbers of galls and egg masses respectively.

galled, whereas no galls occurred on the roots of seedlings in the soil infested with the roots from the *M. hapla* treatments. This was a clear indication that eggs produced at 35°C were viable.

Series 2.—In the 2 tests in this series, tomato plants growing in 0.5-gal crocks were used as host plants. The soil in each crock was infested with 1,000 active larvae of the proper *Meloidogyne* sp. Soil temperatures were 15.6°, 21.1°, 26.7°, 32.2°, and 35°C. Each temperature-species combination was replicated at least 3 times. As indicated previously, the number of root galls, as well as egg masses, was recorded.

In the 1st test, populations 1, 2, and 3 of *M. javanica javanica* and 9 and 10 of *M. incognita acrita* were used (Fig. 2). The population × temperature interactions for numbers of egg masses were significant at the 0.01 level. Population 1 of *M. javanica javanica* produced the greatest number of egg masses at 26.7°C. The number of egg masses produced by population 2 was similar at 21.1° and 26.7°C. Egg-mass production by population 3 was best at 26.7° and 32.2°C. Population 9 of *M. incognita acrita* produced the largest number of egg masses at 32.2°C, whereas population 10 produced the largest number at 26.7°C. At 35°C, population 9 of *M. incognita acrita* produced significantly more egg masses than population 1 (0.05 level) and 2 (0.01 level) of *M. javanica javanica* when compared by the multiple range test of mean differences (3). Differences between other populations were not significant.

In the 2nd test, populations used were 11 and 12 of *M. incognita acrita* and 16 of *M. arenaria thamesi* Chitwood, 1952. Population × temperature interactions were significant at the 0.05 level. Population 12 of *M. incognita acrita* reproduced best at 32.2°C (Fig. 3). Population 11 reproduced best at 26.7° and 32.2°C. *M. arenaria thamesi* showed a lower temperature optimum; the greatest number of egg masses was produced at 26.7° and 21.1°C. All 3 populations produced egg masses at 35°C: population 12 of *M. incognita acrita* produced significantly (0.01 level) more egg masses than *M. arenaria thamesi.* The differences between populations 11 and 12 of *M. incognita acrita* and between population 11 and population 16 of *M. arenaria thamesi* were not significant.

Although there is a correlation between gall formation and egg-mass production, the results (Fig. 2, 3) clearly show that gall production is not necessarily a good measure of reproduction. At 15.6° and 35°C relatively large numbers of galls were produced but few egg masses. Population 9 of *M. incognita acrita* produced many more galls at 35° than any of the other populations; but, as indicated, only produced significantly more egg masses than populations 1 and 2 of *M. javanica javanica.* The largest number of galls produced in the 2nd test in this series was by population 11 of *M. incognita acrita* at 35°C, yet this population produced fewer egg masses at this temperature than population 12 of the same species.

Series 3.—In the 3rd series of tests, *Sesbania* plants were grown in 1-qt plastic pots and the soil in each pot infested with 5,000 larvae. Each temperature-species combination was replicated 4 times in the 1st and 3rd tests, and 3 times in the 2nd test.

In the 1st test, the abilities of population 13 of *M. hapla* and population 7 of *M. incognita acrita* to reproduce at 15°, 20°, 25°, and 35°C were compared (Fig. 4-B). No reproduction of either occurred during the growing period at 15°C. Reproduction of the 2 species was similar at 20° and 25°C. At 30°C *M. hapla* produced more than twice as many egg masses as *M. incognita acrita.* Rate of reproduction of both species fell off markedly at 35°C, but *M. incognita acrita* produced 6 times as many egg masses as *M. hapla.* When the reproduction of the 2 species at 35°C

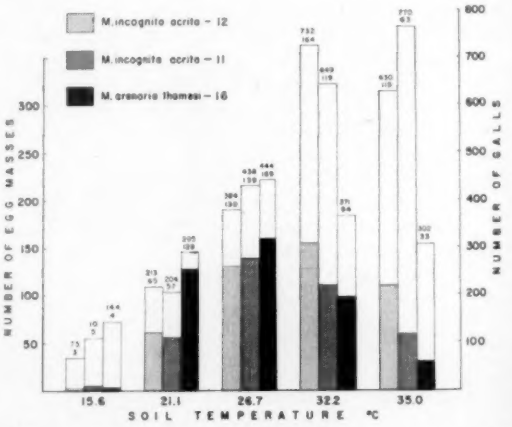

Fig. 3. Influence of soil temperature on root-galling and egg-mass production by *Meloidigyne* spp. on tomato. Bars representing both galls and egg masses have the same base line. Top and bottom numbers above each bar indicate numbers of galls and egg masses respectively.

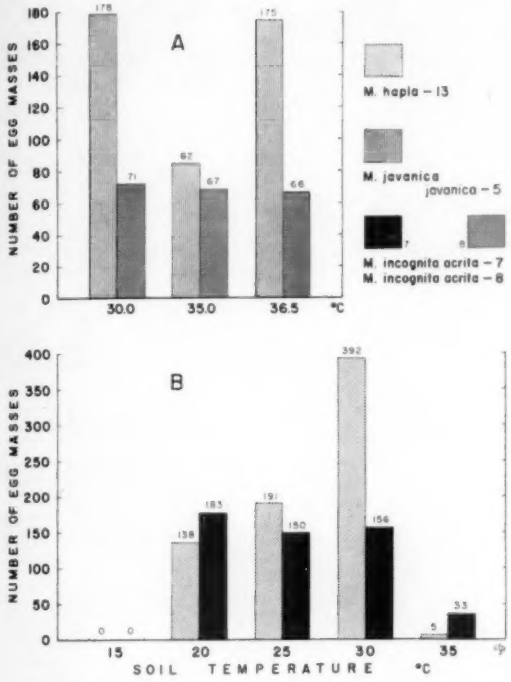

Fig . 4. A-B. Influence of soil temperature on egg-mass production by *Meloidogyne* spp. on *Sesbania exaltata.*

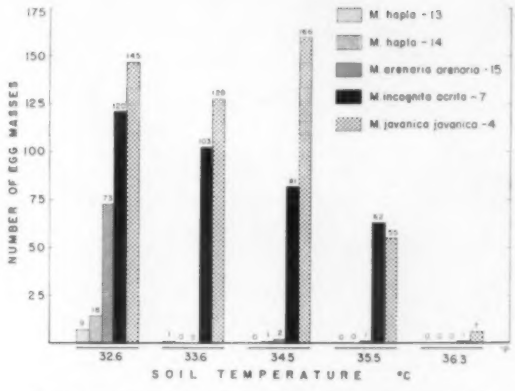

Fig. 5. Influence of soil temperature on egg-mass production by *Meloidogyne* spp. on *Sesbania exaltata.*

was compared by means of the "t" test, the difference was found to be significant at the 0.01 level. There is no apparent reason for the marked increase in egg-mass production of population 13 of *M. hapla* at 30°C and its rapid decline at 35°C.

In the 2nd test in this series, the ability of population 5 of *M. javanica javanica* and population 8 of *M. incognita acrita* to reproduce at 30°, 35°, and 36.5°C was compared (Fig. 4-A). One plant at 35°C in soil infested with *M. incognita acrita* failed to survive to harvest, as did 2 plants in soil infested with this nematode at 36.5°C. Both species produced egg masses at all 3 temperatures. Although *M. javanica javanica* produced more egg masses at all 3 temperatures, the differences were not significant.

In the 3rd test in this series, a more critical temperature range was selected so that the ability of the several species to produce egg masses at extreme temperatures could be evaluated. Populations 13 and 14 of *M. hapla*, 15 of *M. arenaria arenaria*, 7 of *M. incognita acrita*, and 4 of *M. javanica javanica* were tested at 32.6°, 33.6°, 34.5°, 35.5°, and 36.6°C (Fig. 5).

All species produced egg masses at 32.6°C. However, both populations of *M. hapla* produced significantly (0.01 level) fewer egg masses than *M. javanica javanica*, *M. incognita acrita*, or *M. arenaria arenaria*. *M. arenaria arenaria* produced fewer egg masses than *M. javanica javanica* or *M. incognita acrita*, but the difference was not statistically significant. The critical temperature for separating the several species was 33.6°C. At this temperature, *M. javanica javanica* and *M. incognita acrita* continued to produce egg masses at essentially the same level as at 32.6°C, and *M. arenaria arenaria* and the 2 populations of *M. hapla* practically failed. *M. javanica javanica* and *M. incognita acrita* continued to produce egg masses at 35.5°C. A few egg masses were produced in 3 of the 4 replicates of *M. javanica javanica* at 36.3°C.

Discussion.—Apparently some *Meloidogyne* spp. are capable of limited production of eggs at 35-36°C in 35 days. Although there was some variation between tests, the populations of *M. hapla* used differed significantly from those of *M. javanica javanica* and *M. incognita acrita* in their ability to produce egg masses at high soil temperatures. A few egg masses were produced by *M. hapla* populations above 32.6°C, but this temperature appeared to be the maximum at which any consistent egg-mass production occurred during the time allowed in these tests.

Populations within the species *M. javanica javanica* and *M. incognita acrita* appear to differ somewhat in their temperature optima and in their ability to produce egg masses at extreme temperatures. Populations of both species, however, consistently produced egg masses at temperatures above those at which *M. hapla* does, and cannot be separated from each other on the basis of their response to high temperature.

M. arenaria arenaria and *M. arenaria thamesi* fall between the *M. hapla* populations and the *M. javanica javanica-M. incognita acrita* populations. The single population of *M. arenaria arenaria* tested reacted more like the *M. hapla* populations, whereas the *M. arenaria thamesi* population reacts more like the *M. incognita acrita* populations. Additional populations of *M. arenaria arenaria* and *M. arenaria thamesi* would have to be tested before their position in relation to the *M. hapla* and *M. javanica javanica* populations could be adequately determined.

Decreased egg-mass production at the high tempera-

tures could be due in part to a reduction in the number of larvae entering the roots of the host plants. Fig. 2 and 3, however, show that numerous larvae entered the tomato roots at 35°C and incited gall formation, but did not produce egg masses before the tests were terminated. High soil temperatures might also alter the ratio of males to females. Microscopic examination of galls with no egg masses indicated that the majority of the nematodes in them had not developed farther than the spike-tail stage.

Egg production at 35°C is not synonomous with continued reproduction at this temperature. The eggs produced at this temperature must be viable (they were, in 1 test) and capable of maturing and hatching. No tests were conducted to determine whether a 2nd generation would occur at a soil temperature of 35°C. In a hatching test in which egg masses of population 6 of *M. javanica javanica* were incubated in water for 4 and 21 days, the hatch at 33°C doubled from the 4th to the 21st day, but at 36°C it remained the same. The 4-day hatches at 33° and 36°C were similar, indicating that mature eggs containing 2nd-stage larvae were capable of hatching, but that immature eggs were not capable of continued development at 36°C.

The restricted development of *M. hapla* at the high temperatures would suggest that Tyler (7) was working with a population of this species when she reported the maximum temperature for reproduction of root-knot nematodes as 31.5°C.

The lower temperature maximum for egg production by the species *M. hapla* coupled with the ability to survive better at low temperatures (1) adds further support to the contention that *M. hapla* originally developed in areas of cooler climate.

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GIRDLING OF GRAPEVINE CANES BY MELANCONIUM FULIGINEUM

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SUMMARY

Melanconium fuligineum, the pathogen that causes bitter rot of grapes, was found to girdle the base of grapevine canes on 4 varieties of *Vitis vinifera*. The canes may be killed at any time during the vegetative period. A discolored and sunken lesion is produced at the region of girdling. Its surface is speckled with dark masses of spores of the pathogen. Infected berries or parts of clusters were often found on healthy canes early in the season; later, whole clusters were found to be affected.

The pathogen was isolated on culture media and its characters studied. Artificial inoculation of vines through wounds killed the inoculated canes within 1½ months. Artificial inoculations were also made on the unwounded surface of healthy green or ripe berries, and on pedicels and branches of the peduncle. All of these were readily infected and acervuli were produced 5 days after inoculation.

In the spring, diseased canes were derived either from diseased or from healthy arms. Those from the latter became infected while their bark was still green. Diseased canes developed poorly or were killed soon after they became infected. When the pathogen invaded the arm, it was usually killed during the 2nd year after infection. Occasionally all of the arms of a vine were killed.

The girdling of grapevine canes by *Melanconium fuligineum* (Scrib. & Viala) Cav., has been observed to cause considerable damage in vineyards at Temeni, Aigion, Greece. The disease was more prevalent in vineyards where protective sprays against downy mildew were not being applied properly, as could be inferred from the prevalence of that disease. The girdling was observed on Sideritis, Roditis, Razaki, and Savatiano, all varieties of *Vitis vinifera* L.

Bitter rot of grapes caused by the same pathogen

has a limited importance in Greece, apparently because of the protective spray programs used to control downy mildew. Luttrell (1) demonstrated that *M. fuligineum*, besides infecting the berries of both muscadine (*V. rotundifolia* Michx.) and bunch grapes (*V. labrusca* L.), is capable of producing leaf and stem flecks. The fungus was not observed to sporulate on lesions of vegetative parts, and it was so rare and of such little consequence that it was of no practical importance. Luttrell and Murphy (2) stated that the most serious symptoms of bitter rot on muscadine grapes are berry rot and pedicel blight. Flecking was occasional on young leaves and stems. Symptoms of the disease were a similar fleck of sepals and blight of individual flower buds.

MATERIALS AND METHODS.—The pathogen was isolated by transfer of spores or bits of infected bark or wood to potato-dextrose agar (PDA) or cornmeal agar in petri dishes. The cultures were incubated in the laboratory, where temperatures during summer and fall were 22-30°C. The cultures were compared with a pure culture of the pathogen, received from Dr. E. S. Luttrell, Georgia Agricultural Experiment Station, Experiment, Ga., which had been isolated from muscadine grapes in September, 1954.

Artificial inoculations were made on 3 vines of a hybrid horticultural variety growing in pots, which were kept in the open, and 1 vine of the variety Moschato rooted in the ground. Seven canes were inoculated through wounds at the middle of their length with mycelium from PDA of the same isolate. The points of inoculation were wrapped with absorbent cotton and kept moist for the 1st 3 days after inoculation. Uninoculated canes served as controls. The course of the disease was observed for 1½ months.

Detached healthy green berries at the stage before shining, about 1 cm in diameter; and ripe berries of the varieties Sideritis, Fraoula, Razaki, Roditis, Savatiano, Fileri, Siriki, and Monemvassia were inoculated in moist chambers. Healthy unwounded pedicels or branches of the peduncle also were inoculated under the same conditions. Each moist chamber was provided with a disc of blotting paper, which was kept moist for 3 days after inoculation.

The inoculum was prepared with spores taken from a pure culture and suspended in distilled water. Each berry, pedicel, or peduncle branch was inoculated by placing 1 drop of inoculum on the unwounded surface. Infection was allowed to develop at room temperature. The berry inoculation tests were repeated several times.

Symptoms.—The main symptom of the disease in the field was the killing of entire canes. This occurred at any time of the vegetative period, from the end of March, when the shoots were about 15 cm long, to the end of the season. In irrigated vineyards the infected canes died rapidly right after an irrigation.

The canes became infected either on the surface, when the bark was still green, or from within the arm. When the canes were killed at a young stage, the pathogen did not sporulate on them. The death of such canes was due to a girdling that took place farther down the arm and was the result of an infection that occurred the previous year. When older canes were girdled, discolored and sunken lesions were produced (Fig. 1). These were sharply demarcated from the healthy tissue with an irregular advancing margin. Occasionally the bark cracked longitudinally at the girdled region. Acervuli were produced over the surface of the girdled region by the middle of July.

The canker often extended up to 30 cm along 1 side of the cane. On such canes the wood exhibited a brownish discoloration to the upper limit of the canker, but acervuli were produced only at the base. On a cane of the variety Savatiano, lesions were observed at 3 consecutive nodes. Acervuli were produced on each lesion but girdling was incomplete at all 3 nodes. Two of these lesions were at the axils of leaf scars.

On rapidly girdled canes, the withering of the leaves proceeded from the lower to the upper. The lower leaves withered before girdling was apparent. In the variety Sideritis, such leaves withered without turning yellow, and finally became cinnamon-brown. The with-

Fig. 1. Basal portions of canes of Sideritis grapevine showing cankers incited by *Melanconium fuligineum*. The black dots are conidial masses.

ering of a single leaf started from its base and proceeded along the main veins. The withered leaves remained attached to the cane until they were broken off. The leaves exhibited a yellowish discoloration when the lesion occurred on 1 side of the cane.

At the end of September, when the majority of the clusters of the variety Sideritis were still green or slightly pink, the diseased canes could be readily noticed from some distance because the clusters assumed their normal fuchsia color prematurely. At the beginning of November, the dead canes were naked, their dried leaves torn away. The semi-girdled canes of Sideritis, however, were very conspicuous because their leaves exhibited a purple-red discoloration. The leaves of partly broken canes showed the same discoloration.

At the site of infection on semigirdled canes, the base was flat for about 10 cm. On the healthy side it was more or less rounded. Cross sections of half-girdled canes showed that the wood on the side of the lesion was poorly developed. Patches of healthy green bark were left at the infected area in semigirdled canes, although the rest of the cane had the normal brown color.

It was observed that infection could spread from a diseased cane into an arm. The wood cells of such an arm were invaded by the mycelium of the pathogen and became discolored (Fig. 2). In a diseased trunk with a diameter of 5 cm, half of its cross-sectional area was brown. In 1 case the pathogen was isolated early in September from a point of an arm 20 cm below the base of a cane that had become infected the previous year and was not removed by pruning. In another case the fungus was isolated from the edge of the brown area of the wood 75 cm below the girdled region. Under favorable moisture conditions, the pathogen spreads rapidly within the arm before the buds open in the spring. In some vines, arms were killed outright.

The acervuli produced in the girdled region were at first gray-cinereous, then brownish. They were subepidermal and erumpent. The conidiophores were

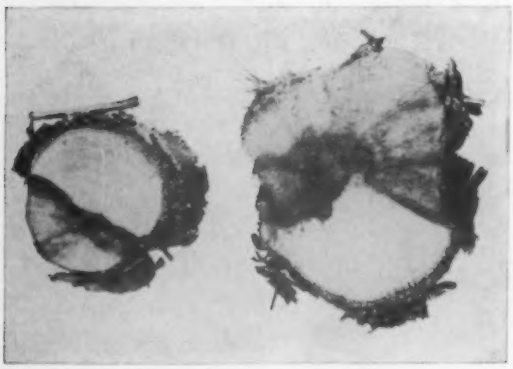

Fig. 2. Cross sections of arms from Sideritis grapevines. The dark discoloration is due to invasion of the pathogen.

Fig. 3. Conidial droplets on a diseased cane of Sideritis grapevine (× 2).

simple, short, and closely packed. The conidia were sooty-colored, 1-celled, ellipsoid to elongated, 6-12 by 2-4 μ, often bent, with unequal diameter, pointed at 1 or both ends, and guttulate. The conidia were produced in droplets over the acervuli (Fig. 3). The spore masses of adjacent acervuli often merged together so that, after dessication, spore masses of various sizes and forms were produced. These larger masses sometimes spread on the surface of the cane forming thin crusts.

Large spore masses on the diseased canes were produced when soil moisture was high at the beginning of summer and late in the fall, after the first rains. On canes that died in the middle of September, none or very few spore masses were produced. This can be explained by the fact that the protracted summer drought in the area continues to the end of September or middle of October.

At the end of August, a few or all the berries of a peduncle branch often were infected; later in the season, whole clusters on healthy canes were affected. It is evident that mycelium spreads within the cluster but often leaves a few berries unaffected. When the peduncle became infected, the cluster often dropped off. In 1 instance the infection proceeded downward from the peduncle into the cane. In spite of production losses, the berry rot was of only minor importance.

The surface of infected berries was densely speckled with acervuli that developed on the stalks as well as on the peduncle and its branches. On infected berries the acervuli first appeared as punctiform, irregular elevations of the epidermis. Upon rupturing, the spore mass turned black. The conidia on ripe berries were produced in droplets as in the girdled region of the canes. Also, the form of the droplets and their dessication were similar. Small tufts of hyphae often protruded from isolated spore masses. Through such hyphal tufts, droplets of liquid often were exuded and became filled with conida. The spore masses were larger on the berries than on the canes.

Results of artificial inoculations.—About 1½ months

after inoculation, all 3 canes of the hybrid variety, and 2 of 4 canes of the Moschato variety died. In 3 of 5 dead canes, the infection spread downward, passing the 1st node. A few acervuli were produced on the internode. The majority, however, were formed at the node and in the region adjacent to it.

Ripe berries in moist chambers became infected when inoculated on the unwounded surface. The varieties Savatiano, Roditis, Fileri, and Monemvassia were most susceptible, acervuli being produced on them 5 days after inoculation. Razaki also was susceptible but the infection proceeded at a slower rate. Sideritis and Fraoula were less susceptible. On the ripe berries of Razaki and Fraoula, there was a sharp demarcation between diseased and healthy tissue. Under similar conditions, green berries of the varieties Sideritis, Siriki, Razaki, Savatiano and Fileri also became infected when inoculated on their unwounded surface; the infection spread to the pedicels.

The infected ripe or green berries assumed a light-brown discoloration but did not shrivel, even when their surface was densely speckled with black masses of spores. The spore masses occasionally formed tendril-like cirri on infected green berries. On uninfected green berries, a superficial brown spot was produced at the point of inoculation. The rot was soft and the fungus sporulated more rapidly in ripe berries. The infected berries ultimately shriveled to hard, dry, black mummies.

Ripe or green berries of all the varieties used became infected when inoculations were made on the uninjured epidermis of the pedicel or peduncle branch. When several berries were attached to the peduncle branch, the infection spread to all of them. In such berries the acervuli were produced first at the pedicel end. The infected pedicels or peduncle branches showed an indefinite brownish discoloration. The fungus sporulated on them 5 days after inoculation. The stalks of unripe Monemvassia berries did not become infected.

Cultural characteristics of the pathogen.—Colonies formed on cornmeal agar plates from either hyphal tip or conidial mass inoculation and kept at room temperature sporulated in 4-6 days, whereas only 24 hours were required for sporulation on PDA plates. Isolates from the varieties Roditis and Razaki behaved exactly alike in the media used.

The mycelium was more or less sparse over the surface of the medium. It was prostrate, light brown, and produced in concentric circles around the point of inoculation. Different isolates varied as to the shade of the light-brown mycelial color; a few of them were light chestnut-violet. In most isolates 1 circle of acervuli was produced around the point of inoculation and the other acervuli were distributed freely. In a few isolates several concentric circles were formed and many acervuli were distributed freely. Isolates sporulated at both the inner and outer edges of the concentric circles of superficial mycelium. In some isolates sporulation was not related to the concentric circles. A limited number of acervuli with spore masses were produced within the medium. Over each acervulus and above the surface of the medium, a droplet of a clear viscous liquid was exuded. This droplet was then gradually filled with spores. When the liquid evaporated, the spore mass became compact and finally dried. A light cinnamon-brown mycelium was occasionally produced on the surface of the spore mass.

It was interesting to compare on culture media the behaviour of these isolates with that of the isolate from Georgia. In the latter, most acervuli were submerged and only a few developed on the surface. Moreover, as many as 6 concentric circles of superficial mycelium were produced and these were not as well defined as with the Aigion isolates.

After 24 hours in a hanging drop of distilled water, about 2% of the spores had germinated. Germ tube length varied 3-42 μ. At germination a single germ tube was produced from any point on the surface of the spore. In the majority of cases, however, it developed from 1 of the ends of the spore. Occasionally, the germ tube was branched.

Spores could withstand a very long dessication. In 1 case the pathogen was recovered from a dry culture plate that had been kept in the laboratory for 1 year. In another instance the fungus was isolated from spores on a cane kept in the laboratory for 1 year. The fungus also was recovered from spores in a pure culture plate kept in a refrigerator for 2 years.

Discussion.—The girdling of grapevine canes by the bitter rot fungus has considerable economic importance in all of the grapevine growing area of northern Peloponnesos. Sideritis was most severely attacked of the varieties observed. The growers have no name for the disease and apparently they confuse it with black measles, which may kill arms or whole vines. The leaf symptoms of that disease are well known to them and when once observed the vines can be saved by successful control measures. The killing of arms or whole vines by *Melanconium fuligineum* is attributed by some of them to black measles that escaped their attention and care. Another confusion may come from the superficial similarity of the acervuli on berries, with the pycnidia which are often produced on berries by *Macrophoma flaccida* Cav.

The infection of new canes takes place either from the surface or from affected canes that have not been removed in pruning. From an externally infected cane, the mycelium spreads through the pith or the wood downward into the arm, which thus becomes partly or entirely invaded. The invaded wood becomes discolored. The pathogen was isolated from the edge of the discolored area as far down as 75 cm below the girdled area. When an arm was partly invaded, girdling was usually completed during the 2nd year and the arm was killed.

Sporulation of the pathogen on the lesions is the main source of inoculum for the infection of healthy canes. It is possible, however, that another source of infection is provided by spores that cling to the bases of diseased canes not removed in pruning, as well as by spores on fallen mummies or nearby pruned canes.

It has been shown through artifical inoculations that infection can take place by placing a drop of inoculum on the unwounded surface of a berry. This would indicate that the fungus spreads by direct contact between diseased and healthy berries if moisture is sufficient for the spores to germinate. Also, the fungus spreads from berry to berry in the cluster through the branches of the peduncle.

The pathogen sporulates 24 hours after a mass of spores is transplanted to a PDA plate. Early production of spores by the pathogen was observed by Timnick, *et al.* (3) following the flooding of maltose ammonium tartrate agar plates with a heavy spore suspension. They observed that the 1st spores were borne directly on the germ tubes and very young mycelium. This early sporulation was explained by the hypothesis that the multiplicity of growing points leads to a depletion of nutrients, which results in an early physiological maturity of the hyphae, and favors the production of spores.

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VISCOSITY OF NAPHTHENIC AND PARAFFINIC SPRAY OILS IN RELATION TO PHYTOTOXICITY AND SIGATOKA DISEASE CONTROL ON BANANA LEAVES

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SUMMARY

A series of 8 saturated naphthenic oils differing in viscosity and a similar series of paraffinic oils were sprayed 4 times at 2-week intervals on banana leaves at a low-volume deposit rate of 2.8 mg of oil/100 cm^2 of leaf area. The oil viscosities in each series varied from about 35 to over 150 SUS at 100°F. At viscosities of approximately 75-85 SUS, both series gave good disease control but the naphthenic oil type caused only 25-50% as much phytotoxic leaf flecking as the paraffinic type. At other viscosities, either disease incidence or phytotoxicity tended to be too high for each oil type. A naphthenic and a paraffinic oil containing 10-12% aromatics and having viscosities of 74-75 SUS also were sprayed on banana leaves. The naphthenic oil gave approximately the same disease control and caused only one-fourth as much leaf flecking as the paraffinic oil. For low-volume spraying on bananas, the naphthenic oils used here were superior to the paraffinic in the 75-85 viscosity range.

Introduction.—Some petroleum spray oils used to control the Sigatoka leaf-spot disease of bananas cause phytotoxicity and are generally believed to lower fruit yields (1, 4, 6). There is a need for oils that control the disease but cause little or no phytotoxicity. In a former study (3), 2 naphthenic and 2 paraffinic oils and their fractions were sprayed on banana leaves to determine which fraction controls Sigatoka and which causes phytotoxicity, measured in terms of leaf flecking. All 4 oils and their saturated fractions controlled the disease, and all caused abundant flecking except for the light naphthenic oil and its saturated fraction. These results suggested that oil type (naphthenic *vs.* paraffinic) and viscosity (a quantitative measure of "lightness") may be important factors in developing better low-volume spray oils for bananas. The present study evaluated the importance of these 2 factors by determining the amount of leaf flecking and Sigatoka control produced by a series of naphthenic and paraffinic oils over a selected viscosity range.

Materials and Methods.—In 1 trial, 16 oils were used that contained no aromatics and had narrow boiling rates. Eight of the oils were prepared by vacuum distillation from a saturated naphthenic oil with a wide boiling range to give a series of oils differing in viscosity. The other series of 8 oils was similarly prepared from a saturated paraffinic oil. The viscosities of the oils in each series varied from about 35 to over 150 Saybolt Universal Seconds (SUS) at 100°F. Some of the oils in these series would fall into the light,

TABLE 1. Certain characteristics of a series of saturated paraffinic and naphthenic oils varying in viscosity

Oil type and viscosity (SUS at 100°F)	Distillation range °F[a]					Neutralization number[b]	Specific gravity (60/60°F)	Refractive index (20°C)
	Initial	10%	50%	90%	Complete			
Paraffinic								
35	430	460	489	593	667	0.01	0.800	1.4421
41	472	520	591	653	728	0.03	0.815	1.4477
47	538	586	622	698	751	0.01	0.824	1.4538
54	584	615	652	719	763	0.01	0.831	1.4569
64	608	648	691	747	791	0.02	0.841	1.4612
78	653	688	722	765	804	0.01	0.847	1.4629
96	693	722	745	781	818	0.03	0.851	1.4668
152	716	755	784	832	870	0.03	0.860	1.4692
Naphthenic								
36	456	472	482	502	520	0.06	0.848	1.4610
46	532	560	579	604	629	0.07	0.851	1.4650
52	586	610	623	637	658	0.09	0.863	1.4697
68	602	646	654	669	681	0.10	0.865	1.4703
82	658	675	684	695	707	0.06	0.867	1.4710
103	686	703	708	721	735	0.06	0.868	1.4732
144	713	734	738	752	797	0.06	0.871	1.4786
252	761	773	780	792	795	0.05	0.878	1.4840

[a] Converted to 760 mm Hg from 10 mm data.
[b] mg KOH/g of oil.

medium, or heavy grade categories used for agricultural spray oils on other crops (5). Table 1 lists certain characteristics of the 16 oils in this trial.

Another trial compared 1 naphthenic and 1 paraffinic spray oil containing aromatics. The characteristics of these 2 oils are given in Table 2. The selection of 2 oils with these characteristics was influenced by the results from the above trial with saturated oils as shown later.

The naphthenic oils used in this study and in a previous one (3) came from a single area in Texas, while the paraffinic oils came from the Louisiana-Mississippi area. Oils from other areas may differ from the above oils in their degree of "naphthenicity" or "paraffinicity" since these are only relative terms.

Each oil was applied to the upper surface of Dwarf Cavendish banana leaves in the field by a precision spray technique described previously (2). The deposit used was 2.8 mg of oil/100 cm^2 of leaf surface. Each treated leaf received a total of 4 sprays at 2-week intervals; the 1st application was made when the unfurled leaf was less than 1 week old. The deposit rate and spray cycle were comparable to commercial spray practices. The right half of each leaf was sprayed; the left half was kept as an unsprayed check. Additional checks consisting of unsprayed whole leaves were used as discussed previously (3). Each spray material was applied on 8 replicate leaves. The treatments were arranged in a randomized block design.

Observations on phytotoxicity and disease incidence were made 2 weeks after the final spray. Flecking was the only phytotoxic symptom observed on the treated leaves. Phytotoxicity was evaluated as the percentage of sprayed leaf area covered with these flecks. Disease incidence on each leaf was evaluated as the percentage of sprayed leaf area with disease (right half) divided by the percentage of unsprayed leaf area with disease (left half). A low disease rating indicates low disease incidence.

RESULTS.—*Comparison of saturated naphthenic and paraffinic oils at several viscosity levels.*—The phytotoxicity and disease incidence results are presented in Fig. 1. Each point represents the average value for 8 replicate leaves. The standard error, if other than 0, for each average is indicated by the vertical lines drawn through the points.

In the naphthenic oil series, disease incidence was high at viscosities of 36, 45, and 52 SUS; intermediate at 68; very low at 82; and very low at higher viscosities. The percentage of flecking, on the other hand, was 0 at 36 and 45 SUS; low at 52 and 68; intermediate at 82 and 103; and high at 150 and 250 SUS.

TABLE 2. Certain characteristics of a naphthenic and paraffinic spray oil containing aromatics

Characteristic	Naphthenic oil	Paraffinic oil
Viscosity, SUS at 100°F	74	75
Distillation range, °F[a]		
Initial	562	646
10%	612	682
50%	664	708
90%	719	743
Complete	764	789
Unsulfonated residue	95	94
Oil composition[b]		
Saturates, %	87.3	89.2
Aromatics, %	12.3	10.7
Nonhydrocarbons, %	0.4	0.1
Neutralization number[c]	0.06	0.03
Specific gravity, 60/60°F	0.874	0.850
Refractive index, 20°C	1.4750	1.4670

[a] Converted to 760 mm Hg from 10 mm data.
[b] By silica gel chromatography.
[c] mg KOH/g of oil.

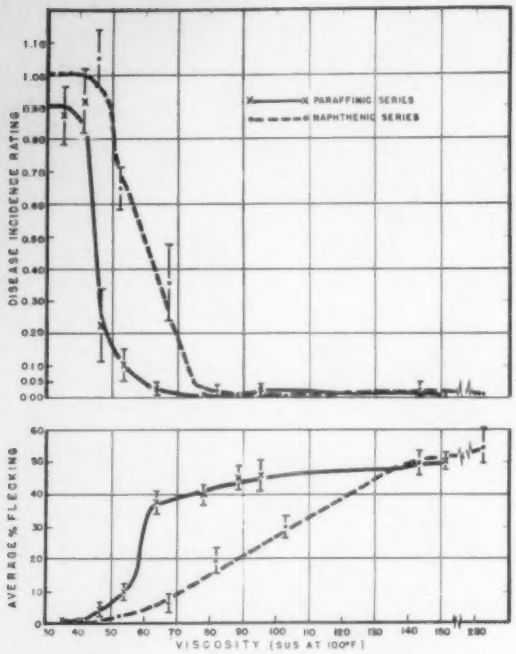

Fig. 1. The amount of phytotoxicity and Sigatoka disease on banana leaves sprayed with either saturated naphthenic or saturated paraffinic oils of different viscosities. The standard error, if other than 0, is indicated by vertical lines through the data points.

In the paraffinic oil series, disease incidence was high at 35 and 41 SUS; intermediate at 47 and 54; very low at 64; and very low at higher viscosities. Percentage of phytotoxic flecking, on the other hand, was 0 at 35 and 41 SUS; low at 47 and 54; high at 64; and then leveled off.

When disease incidence is at its lowest level (under 0.05), the naphthenic phytotoxicity curve is significantly lower than the paraffinic curve in the viscosity range of about 75-110 SUS. At higher viscosities, the amount of flecking from either oil type is high and about the same. The naphthenic flecking curve is around 25 and 50% of the value of the paraffinic curve at 75 and 85 SUS, respectively. At the best levels of disease control, therefore, the naphthenic oil type, especially from 75-85 SUS, seems better suited for spraying bananas than the paraffinic oil type.

Comparison of aromatic-containing naphthenic and paraffinic oils at 1 viscosity level.—It was just seen that saturated napthenic oils gave less phytotoxic flecking than saturated paraffinic oils at several viscosity levels. Commercial spray oils being used on bananas, however, are not saturated oils, since they do contain at least 5-10% aromatics as determined by silica gel chromatography. (Personal communication from the Esso Research and Engineering Co.) The presence of aromatics in the spray oil may alter the apparent advantage of a naphthenic oil over a comparable paraffinic oil. In the present trial, therefore, naphthenic and paraffinic spray oils that were similar in aromatic content and viscosity (Table 2) were compared for their effect on banana-leaf flecking and Sigatoka disease control.

The 94-95% unsulfonated residue of these 2 oils is typical of a good agricultural spray oil (5). The viscosity level of 74-75 SUS was chosen, since at this level in the trial with saturated oils the naphthenic curve indicated only 25% as much flecking as the paraffinic curve, whereas disease control was good for both oil types.

The results from the aromatic-containing oils showed that the amount of phytotoxic flecking was 4.5 and 20.7%, respectively, for the naphthenic and paraffinic types. The naphthenic figure is about 25% of that of the paraffinic, which is in agreement with the phytotoxicity curve of the earlier trial at around 74-75 SUS. The absolute values, however, were lower in the present trial. This difference was probably due: 1) to seasonal effects, since the 2 experiments were conducted at different times of the year, and 2) to the dilution of the active saturates in the oil by the presence of 11-12% of aromatic fraction, which is inactive at these application rates (3).

The disease incidence ratings were 0.11 and 0.16, respectively, for the paraffinic and naphthenic oils. The paraffinic rating is slightly lower than the naphthenic, and this relationship also occurs in the disease incidence curves at 74-75 SUS in the earlier trial with saturated oils. The absolute ratings differ between the 2 experiments, and this difference is probably influenced by the same factors mentioned in the preceding paragraph.

The important point emerging from these results is not the absolute levels of flecking and disease incidence, but the fact that aromatic-containing naphthenic and paraffinic oils showed the same relative difference in performance as the saturated oils. The naphthenic oil with aromatics caused far less phytotoxic flecking than the comparable paraffinic, and both oils gave about the same amount of disease control. It appears, therefore, that the presence of around 12% aromatics in a spray oil does not markedly alter the superiority of the naphthenic oil type over the paraffinic for use on banana leaves.

Discussion.—An earlier report (3) explored the effect of chemical composition of oils on banana leaves and suggested that a light naphthenic spray oil would give good Sigatoka disease control and cause less phytotoxic flecking than a paraffinic oil. The present study on viscosity adds further evidence that the naphthenic oil is superior to the paraffinic oil, particularly in the viscosity range of 75-85 SUS at 100°F. Furthermore, the presence or absence of an aromatic fraction apparently does not alter the relative performance of the naphthenic oil compared with the paraffinic.

The effect of other factors will have to be investigated before the characteristics of the best oil for

low-volume spraying of bananas can be determined. The present study does show, however, that the naphthenic spray oils tested here are superior to the paraffinic oils over a definite viscosity range.

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AN ANOMALOUS EFFECT OF PENTACHLORONITROBENZENE ON THE INCIDENCE OF DAMPING-OFF CAUSED BY A PYTHIUM SP.

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SUMMARY

Pentachloronitrobenzene applied as a soil fumigant reduced damping-off of *Pinus radiata* and *P. patula* seedlings caused by *Rhizoctonia solani*. Similar soil treatment was followed by increases in damping-off caused by a *Pythium* sp. The latter effect has been shown to be due to a selective action against the competitors of the *Pythium* sp. in the soil, 1 of which has been identified as *Penicillium paxilli*.

INTRODUCTION.—Pentachloronitrobenzene (PCNB) has previously been effective as a soil fumigant against *Rhizoctonia solani* Kühn but of little use against *Pythium spp.* (1, 3, 5). This has been confirmed in recent experiments on control of damping-off in pine seedlings by East African isolates of *R. solani* and a *Pythium sp.* The latter has not been identified farther than the genus, due to its failure to form a sexual stage in culture; it has been deposited at the Commonwealth Mycological Institute under No. IMI 78272.

Results from experiments with the *Pythium* sp. showed that not only did PCNB fail to control disease caused by the fungus, but also that appreciable increases in disease followed use of the fungicide. This was investigated further in laboratory experiments.

MATERIALS AND METHODS.—Five initial trials of PCNB were made; 4 were located in the glasshouse (18-29°C) and 1 in a well-lit, ventilated shed (13-21°C). All these experiments were based on plot units of 6-in. diameter, porous, earthenware pots filled with sieved local forest soil. Inoculum of the pathogens was grown in 250-ml flasks containing 100 g of sterile corn meal sand mixture (3 g corn meal, 13 g water, 84 g sand) which were incubated at 25°C for 10 days and stored in the refrigerator. This inoculum was added to soil in experiments where appropriate at a rate of 30 g/pot, spread on the soil surface, and worked into it to a depth of about 0.5 in. PCNB was applied where required as a 20% dust formulation at 2 oz/yd^2 of soil and worked into the top 0.5 in. of soil 24 hours after the application of inoculum, just before seeds were sown. *Pinus radiata* D. Don and *P. patula* Schl. & Cham. were used in experiments; uniform counted numbers of seeds were sown in each pot. The seeds of *P. patula* germinated in laboratory tests at 85% and those of *P. radiata* at 75-80%. After sowing, the seeds were covered with enough sand to prevent their disturbance in the course of daily watering. Pots were counted for totals of emerged healthy and diseased seedlings 10 days after emergence; diseased seedlings were sampled at the same time and plated out to check the identity of the pathogen. The latter was done by laying out clean sections of diseased stems, about 1 cm long, on the surface of sterile plates of 2% malt agar and incubating them for 48 hours at 25°C before examination. Randomized block designs were used in experiments in which various combinations of fungicide, inocula, and pine species were

TABLE 1. Effects of PCNB soil treatment on incidence of pine damping-off caused by *Rhizoctonia solani* and a *Pythium* sp.

Experiment No.	Test species	Total seeds sown/treatment		Inoculum added: *R. solani* PCNB	*R. solani* NIL	*Pythium* sp. PCNB	*Pythium* sp. NIL	Nil PCNB	Nil NIL
1.	*Pinus patula*	900	[a]	667	198	....	....	630	558
			[b]	0.5	43.2	....	....	0	4.2
2.	*P. patula*	1800	[a]	....	....	780	899	863	1059
			[b]	....	....	7.9	0.2	11.0	0.6
3.	*P. patula* *P. radiata*	900	[a]	515	132	380	758	....	....
4.	*P. patula*	1500	[a]	1215	195	650	1055	1190	1060
			[b]	4.9	25.6	32.1	12.6	5.5	3.4
5.	*P. patula* *P. radiata*	1400	[a]	....	....	1028	1183	829	1159
			[b]	....	....	21.7	0	17.1	0

[a] Total healthy seedlings harvested.
[b] Percentage of postemergence damping-off.

tested, replicated 4-6 times. Analysis of the results of these experiments was made using figures for total numbers of healthy seedlings at time of final record.

Later experiments to determine the effect of PCNB on the soil microflora were based on plate cultures grown on Czapek Dox agar to which 0.05% yeast extract had been added. This medium was used for the plating of soil dilutions and for growth studies of fungi in pure culture. Soil suspensions were plated out at a dilution of 1:10,000 after making up in sterile distilled water. Cultures were incubated generally at room temperature under bell jars (17-21°C). PCNB was applied to cultures in its vapor phase by placing 0.5 g of the 20% formulation used in pot experiments in the lid of an inverted culture plate; groups of plates treated in this way were incubated under bell jars. The investigations of Reavill (4) indicate that this dosage of PCNB would be sufficient to saturate the air over the cultures with vapor.

RESULTS.—The results of the pot trials are given in Table 1. In all experiments where *R. solani* was used as inoculum, disease losses caused by this fungus were reduced by a statistically significant margin by PCNB. Conversely, the action of PCNB on disease caused by the *Pythium* sp. increased this to a statistically significant degree, largely in the postemergence stage. Indeed, in 2 experiments, the pathogen changed from causing negligible damage to moderate or severe damage. In experiments where no inoculum was added to a portion of the pots, increases in disease following PCNB treatment also were observed (Experiments 2, 5). Where this happened, a *Pythium* sp., similar to that used for additional inoculum, was isolated from diseased tissues. The effects of the fungicide were constant, irrespective of test plants or site of experiment. No phytotoxic effects of PCNB toward pine seedlings were noted in the course of experiments.

A check on the effects of PCNB vapor on the growth of the pathogens in culture confirmed the results of a number of previous workers (1, 3, 5) that *R. solani* was very sensitive to this fungicide whereas the *Pythium* sp. was only slightly affected. (Table 3 confirms the latter finding.) Apparently, stimulation of pathogenicity of the *Pythium* sp. by PCNB might be due to action of the fungicide on a competitor to the spread of the pathogen through the soil.

In the 1st experiment, soil suspensions were set up in 12 plates, half treated with PCNB vapor and half untreated. When these plates had been incubated for 3 days, fungus colonies were evident. At this point, inocula of *Pythium* were introduced on the side of each plate. Growth was observed for the next 3 days. At the end of this period, the greatest radial growth of *Pythium* on each plate was measured and a note was made of the number of points at the edge of the colony where growth had been inhibited by members of the soil microflora. Total fungal and bacterial colonies after 6 days were noted, the former being broken down into 2 size classes.

The results of this experiment (Table 2) show *Pythium* made freer growth through soil plates over PCNB vapor than through those untreated. Further, counts of colonies arising from the soil suspension

TABLE 2. Effects of PCNB vapor on the growth of fungi and bacteria from a plated soil suspension and the effect of these on the growth of a pathogenic *Pythium* sp.

Treatment	Avg. no. of colonies/plate after 3 days incubation[a]: Fungal colonies over 0.5-in. diameter	Fungal colonies under 0.5-in. diameter	Bacterial colonies
NIL	0.8 (0,2)	9.2 (3,14)	13.8 (10,18)
PCNB	0.7 (0,2)	5.7 (1,11)	9.2 (4,16)

Treatment	Growth of *Pythium* sp. on same plates between 3rd and 5th days of incubation: Maximum radius of growth (mm)	Number of points at colony margin where growth was inhibited
NIL	14.2 (0,26)	1.4 (1,3)
PCNB	21.8 (20,24)	0.8 (0,2)

[a] All figures show means of 6 replicates (with maximum and minimum records for individual plates in parentheses).

TABLE 3. Effects of *Penicillium paxilli* on the growth of a *Pythium* sp. in culture in the presence and absence of PCNB

Radius of growth of *Pythium* sp. after 5 days (mm)		
Presence of *P. paxilli*	Presence of fungicide	
	NIL	PCNB
NIL	56	59
Isolate 7	30,23[a]	46,47
Isolate 2	35,35[a]	36,39
Isolate 5	36,40[a]	47,49

[a] Growth stopped between 3rd and 4th days.

indicated that the PCNB apparently had its greatest inhibiting effect on the slower-growing fungi and possibly the bacteria. This experiment was repeated with greater replication and the same results, and the added observation that the growth of the *Pythium* was inhibited in the great majority of cases in the vicinity of colonies of *Penicillium* spp. Seven random isolations of such *Penicillia* were identified at the Commonwealth Mycological Institute as *Penicillium paxilli* Bain.

Three of these isolates were tested as antagonists to the growth of the *Pythium* sp. by sowing a spore suspension at 1 side of a culture plate opposite a point where a piece of *Pythium* sp. inoculum had been sown. Subsequent measurements made on these plates showed that growth of the *Pythium* stopped not less than 1 cm from the region colonized by the *P. paxilli*. This experiment was repeated with half the plates incubated over PCNB. Results (Table 3) show that, in the absence of the fungicide, growth of the pathogen was inhibited by *P. paxilli* after 3-4 days; in the presence of PCNB vapor, growth continued until after the 5th day when the test plates were nearly covered.

DISCUSSION.—The results of this investigation have indicated that the increased activity of a *Pythium* sp. (IMI 78272) as a pathogen following soil treatment by PCNB may be due, at least in part, to the suppression of the growth of *Penicillium paxilli* in the soil by the fungicide. This fungus occurred commonly in the soil under investigation and inhibited growth of the pathogen in its vicinity.

Previous workers have indicated that undesirable results such as this may follow the use of highly selective fungicides in the soil (2), but it is thought that this is the 1st example following use of PCNB.

It is possible that soil inoculum of *P. paxilli* might be used to control damping-off in soils heavily infested with *Pythium* spp. This has been tried, however, in 2 cases using local nursery soils with a history of disease caused by *Pythium* spp.; results were inconclusive. It would appear that this is an unlikely practical extension of the present work.

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PEROXIDASE ACTIVITY IN VIRUS-INFECTED SWEET POTATOES

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SUMMARY

Peroxidase activity was investigated in veinclearing virus infected sweet potatoes. In infected plants, peroxidase activity of both leaves and roots was significantly higher than in healthy ones. Increase in peroxidase activity following inoculation commences with the appearance of symptoms in the infected plants. The activity varies with age of leaf, hour of daily measurement, and age of infection. The increase seems to be associated with presence of the virus in the tissue *per se*.

INTRODUCTION.—Among the physiological effects of virus infections on host plants, changes were observed in over-all respiratory activity (12). Very little work, however, has been done on the specific oxidative enzyme systems of virus-infected plants (3, 11).

Changes in activity of several oxidative enzymes in fungus-diseased plants were found by many workers, as reviewed by Kiraly and Farkas (4).

In a recent study (5) on sweet potatoes, respiration rates of veinclearing infected plants were significantly

higher than those in healthy plants. The absolute values were dependent on leaf age, hours of daily measurement, and interval after infection. Veinclearing virus causes severe reductions in yields of sweet potatoes in Israel (6).

Exploratory investigations of several oxidative enzymes in veinclearing infected sweet potatoes showed especially pronounced alterations in peroxidase activity. Changes in peroxidase activity in white potatoes infected by potato virus X also were noted by Umaerus (9). In view of this, more detailed studies of this enzyme system in veinclearing infected sweet potatoes were undertaken.

MATERIALS AND METHODS.—Healthy and veinclearing virus infected sweet potatoes, variety No. 21 (B-52) were pot-grown in a screened greenhouse. All plants came from healthy stocks grown for over 3 years in our greenhouses; the plants were tested, as described in a previous report (6), apparently free from virus infections. During the course of the experiments, plants were given a complete, balanced nutrient solution at 2-week intervals and the usual precautions were taken against accidental infections. Infection was induced by grafting with veinclearing infected scions. Infected plants chosen for assay always showed pronounced veinclearing (6). Leaves were sampled by cutting out discs with a sharp 1.5-cm corkborer. Discs were immediately immersed in 10 ml distilled water for homogenization (as a rule, 6 discs/sample gave sufficient activity for accurate determination). Roots were sampled by cutting approximately uniform segments, weighing about 200 mg, and immersing them immediately in water. Homogenization was carried out as soon after cutting as possible.

Samples were ground in 10 ml of cold, distilled water by a Virtis "45" homogenizer at 45,000 rpm and rapidly filtered through gauze or cotton wool. The homogenate could be stored in an ice water bath for as long as 24 hours before assaying.

For estimating peroxidase activity, a colorimetric assay, developed by A. Cohen (personal communication) and based upon other methods (2), was employed.

Reagents.—1) H_2O_2-1%; 2) Pyrogallol reagent—made up immediately before use from a) pyrogallol stock solution, 0.5 *M* and b) phosphate buffer, pH 6.0, 0.066 *M*. Ten ml A and 12.5 ml of B were mixed and made up to 100 ml with distilled water. All solutions were refrigerated.

PROCEDURE.—To 5 ml of pyrogallol reagent in colorimeter test tubes was added 0.1-0.5 ml (depending upon activity) of crude homogenate; the tube was inverted to mix contents, then inserted in a Bausch and Lomb colorimeter at 420 mμ. The colorimeter galvanometer was then adjusted to 0 optical density (O.D.); 0.5 ml of 1% H_2O_2 was added rapidly to the tube. The tube was inverted once, immediately re-inserted in the colorimeter, and timed by stopwatch between O.D. 0.10 and 0.30. With the enzyme concentrations used, reaction times generally varied 10-60 seconds. As there is no appreciable decomposition of peroxidase in such short periods, enzyme activity can be assumed inversely proportional to the time required for the change in opical density (*i.e.*, the higher the activity, the less time required to reach the designated optical density).

Absolute peroxidase values for each sample were expressed either as $(\text{sec} \cdot \text{mg fresh wt})^{-1}$ or $(\text{sec} \cdot \text{cm}^2 \text{ leaf area})^{-1}$. For comparison of peroxidase activity between infected and healthy plants, the ratio of $\frac{\text{absolute activity infected}}{\text{absolute activity healthy}}$ was made; this is referred to as "relative activity" of the infected sample with the relative activity of the healthy control taken as 1.0).

RESULTS.—*Average peroxidase values in sweet potato leaves.* Six diseased plants and 6 healthy ones were selected. One disc leaf, 2nd-7th open leaves, was taken from each plant, weighed, homogenized, and assayed. The results, calculated on both a fresh weight basis and leaf area basis, are given in Table 1. In infected plants, peroxidase activity is significantly higher than in healthy ones. In a 2nd series, similar results were obtained. No significant differences in relative activity were found between results on leaf area basis and fresh weight basis. It was therefore decided to express results on an area basis in those cases in which only leaf activity was studied.

Activity in roots and leaves.—Roots and leaves of 3 infected and 3 healthy plants were assayed simultaneously. Leaf discs were sampled and weighed as previously (Table 2). In both roots and leaves, a significantly higher peroxidase activity was found in infected plants than in healthy ones. While the results show a higher absolute activity in roots than in leaves, in both healthy and infected tissue, the difference between healthy and infected is more pronounced in leaves. In view of this, it was decided to concentrate mainly on leaf studies.

Influence of leaf age.—Six healthy and 6 infected plants were selected. One disc was taken from the 1st open leaf (youngest) of each plant, combined, homogenized, and assayed. The same was done with the 2nd-7th leaves (Fig. 1). In general, less peroxidase activity was found in younger leaves than in mature ones, either healthy or infected. These results were confirmed by other experiments in which the 1st open leaf was in a very immature stage; the peroxidase level was often less than half of older leaves. In all leaf stages, peroxidase activity was significantly higher in infected than healthy plants.

TABLE 1. Mean peroxidase activity of healthy and veinclearing infected sweet potato discs

	Fresh weight basis		Leaf area basis	
	$(\text{sec} \cdot \text{mg})^{-1}$	Relative activity	$(\text{sec} \cdot \text{cm}^2)^{-1}$	Relative activity
Healthy	3.12×10^{-3}	1.0	4.40×10^{-2}	1.0
Infected	6.48×10^{-3}	2.08	9.03×10^{-2}	2.05
L.S.D.[a]	2.07×10^{-3}		3.40×10^{-2}	

[a] Least significant difference at P 0.05.

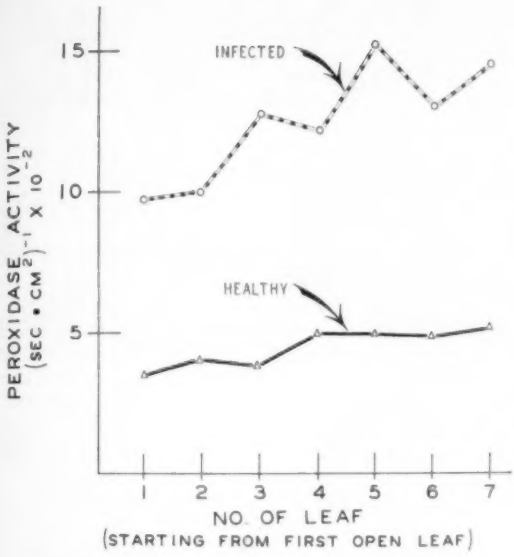

Fig. 1. Peroxidase activity in healthy and virus infected sweet potato leaves of different ages.

Diurnal variation.—Preliminary experiments showed that cutting out discs from a leaf had no apparent effect on peroxidase activity of adjacent areas on the same leaf so a number of samples were taken from the same leaf. From each of 6 healthy and 6 infected plants, 1 disc/leaf was taken from the 2nd-7th open leaves, homogenized, and assayed as usual. Samples were always taken from the same leaves (left on the plants) at 8 AM, 12 noon, 4 PM, and 8 PM (Fig. 2). A rise in absolute activity, reaching a maximum in the afternoon and dropping after dark, can be seen for both healthy and infected plants. The rise is particularly noticeable in the infected plants. Relative activities of 2.30 at 8AM, 2.62 at 12 noon, 2.73 at 4 PM, and 2.49 at 8 PM were calculated. In a similar experiment, activities at 12 noon and 12 midnight were observed. The results were similar; *i.e.*, there was a higher relative activity at noon (2.74) than at midnight (2.19).

Activity increase following infection.—Twelve healthy plants were selected. Six were grafted with veinclearing scions and the other 6 (controls) were grafted with healthy scions. Samplings were made regularly at 4-5-day intervals, up to 30 days after infection, by taking 1 disc/leaf from the 2nd-7th open leaves of each plant. Leaves were left on the plants and the same plants taken for each sampling. Results, expressed as relative activity, are given in Fig 3. Earliest symptoms appeared about 12-15 days after infection, coinciding with the 1st noticeable rise in relative peroxidase activity. In other samples of plants infected 2-3 months, relative activities as high as 3.6 were often found.

Local lesion virus infections.—Veinclearing virus infection in sweet potatoes is systemic. As we were interested in whether the increase in peroxidase activity of a given plant tissue upon infection depends on presence of virus in the tissue itself, or whether it occurs in uninfected tissue as a systemic effect, we chose a local lesion virus, potato virus X (PVX) in *Gomphrena globosa* L. for exploratory investigations. Ten mature healthy *Gomphrena* plants were selected. The lower leaf pairs of 5 plants were inoculated with PVX. Corresponding lower leaves of 5 controls were rubbed with water. After 10 days, when lesions were prevalent, discs were taken both from the lower leaves of each group and the corresponding, untreated upper leaves of the same plants (Table 3). A conspicuous increase in activity was found only in the inoculated leaves. No significant change was observed in the upper, uninoculated leaves of PVX infected plants. Therefore, activation of peroxidase seems nonsystemic; it probably is confined to virus infected cells.

TABLE 2. Peroxidase activity in roots and leaves of healthy and veinclearing infected sweet potatoes

	Roots		Leaves	
	$(\text{sec} \cdot \text{mg})^{-1}$	Relative activity	$(\text{sec} \cdot \text{mg})^{-1}$	Relative activity
Healthy	5.70×10^{-3}	1.0	2.68×10^{-3}	1.0
Infected	9.80×10^{-3}	1.7	8.76×10^{-3}	3.3
L.S.D.	3.89×10^{-3}		5.86×10^{-3}	

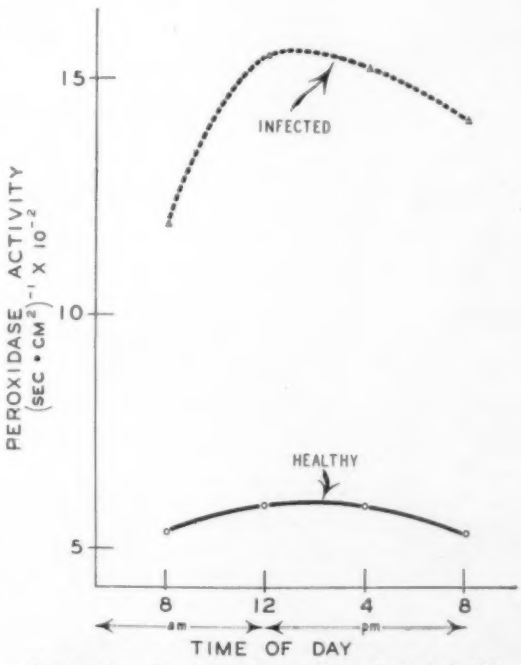

Fig. 2. Diurnal variation of peroxidase acitvity in healthy and veinclearing virus-infected sweet potato leaves.

DISCUSSION.—Apparently there is a positive relation between peroxidase activity and development of veinclearing virus infection. Increase in relative peroxidase activity began when symptoms appeared and was found only in infected tissue, so it seems this effect depends upon the presence of the virus in an active state in the plant cells.

A comparison of peroxidase activity with total respiration (as measured by O_2 consumption) in veinclearing infected sweet potatoes (5) shows several marked differences.

1) The relative peroxidase activity kept rising with age of infection and was often as high as 3.6 in old infected plants; whereas the relative respiration rate leveled off to about 2.0 after a 1-month infection.

2) Peroxidase activity was lower in young leaves than mature ones in both healthy and infected plants whereas total respiration rates were higher in young leaves than mature ones.

3) The diurual deviation in peroxidase activity was much higher for infected than healthy plants whereas diurnal deviation in total respiration ran parallel in both healthy and infected plants.

This suggests that peroxidase may be involved in a different oxidative system than that primarily responsible for total respiration values, usually assumed to be the glycolytic pathway (1, 10), and that this system becomes activated in virus infections.

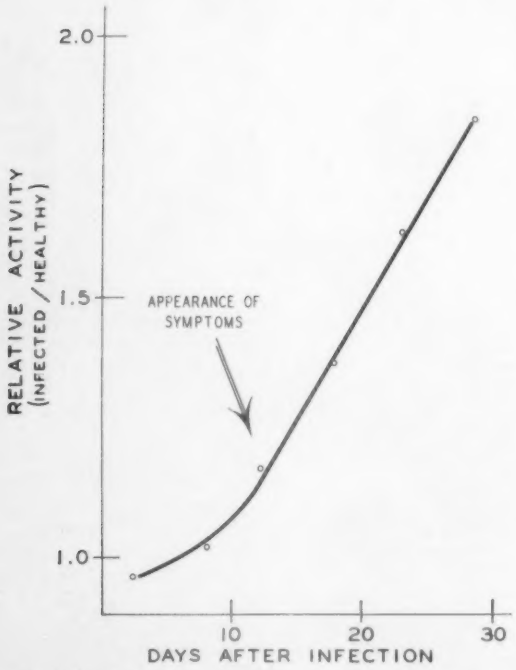

Fig. 3. Increase in relative peroxidase activity of sweet potato leaves after infection by the veinclearing virus.

TABLE 3. Peroxidase activity in leaves of healthy and potato virus X infected *Gomphrena globosa* plants in $(sec \cdot cm^2)^{-1}$

	PVX infected plants	Controls
Upper leaves (untreated)	1.88×10^{-2}	1.75×10^{-2}
Lower leaves (treated)	11.30×10^{-2}	1.58×10^{-2}

As yet, the physiological role of peroxidase, even in normal metabolism, is not clearly understood. It is known to catalyse the oxidation of many mono- and di-phenols, and aromatic amines to quinones in the presence of hydrogen peroxide (1). It may not, however, function as a terminal oxidase in a respiratory mechanism. Some indirect evidence for a dehydrogenase-like action of peroxidase in horseradish has been reported (7). It has also been suggested that the terminal oxidase in cabbage infected by *Botrytis cinerea* Pers. is a flavoprotein system, producing H_2O_2 which is removed by peroxidase (8).

Possibly peroxidase is connected with the hexose monophosphate shunt (HMP) respiratory pathway, in which glucose-6-phosphate is dehydrogenated to 6-P-gluconate and other intermediates such as ribose-5-P or xylulose-5-P. As plant tissue ages, this pathway tends to replace the glycolytic one (10). It also may be active in a number of fungus infections (10). Intermediates of the HMP pathway have been found as precursors in the biosynthesis of aromatic compounds in plants, such as coumarin, phenylalanine, and polyphenols (10). If peroxidase were to function as an oxidase of aromatic compounds produced in the HMP pathway, much of its activity in veinclearing infected sweet potatoes could thus be explained.

The simplicity of the peroxidase assay and the sensitivity of peroxidase activity to those virus infections in which it has been investigated could make this assay a promising technique for studying the comparative physiology of other virus diseases.

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RACE GROUPS OF TILLETIA CARIES AND TILLETIA FOETIDA FOR VARIETAL-RESISTANCE TESTING

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SUMMARY

The presently recognized 28 races of the common bunt fungi *Tilletia caries* and *T. foetida* have been reclassified into 17 pathogenic types on the basis of the differential reactions of 7 wheat varieties which exploit current information on varietal resistance. These 17 pathogenic types are further classified into 6 groups on the basis of their pathogenicity against 1 of the 6 generally recognized resistance types. This proposed system of race classification is designed primarily to facilitate testing for varietal resistance to the bunt fungi.

INTRODUCTION.—Since the 1st report (4) of pathogenic specialization in the bunt fungi *Tilletia caries* (DC.) Tul. and *T. foetida* (Wallr.) Liro, many contributions have been made toward a standard race classification in relation to breeding resistant wheat varieties (5, 10, 11, 12, 13, 14, 15). The race classification developed by Rodenhiser and Holton (13) has been the standard in the United States for 2 decades. Since 1920, inheritance studies have led to the identification of general types of and specific genes for smut resistance in wheat (1, 2, 3, 6, 7, 8, 9, 16). The results of these studies, which would greatly facilitate the varietal-resistance testing program, are not fully utilized in the present system of race classification. This paper proposes a reclassification of the known races of *T. caries* and *T. foetida* based on the differential reactions of 7 host tester varieties which exploit current varietal-resistance information.

MATERIALS AND METHODS.—Spores used in this study were from stock spore collections of 27 races of *T. caries* and *T. foetida* which have been maintained and replenished periodically over the past 20 years by the Regional Smut Research Laboratory. The differential varieties used were Albit C.I. 8275, Selection 50077 C.I. 13561 (Martin C.I. 4463 × Elgin C.I. 11755) (16), Selection 1403 C.I. 13562 (Hussar C.I. 4843 × Hard Federation C.I. 4980) (1), Orin C.I. 12687, Hohenheimer C.I. 11458, Ridit C.I. 6703, and Omar C.I. 13072. Hybrid 128 C.I. 4512 was included as a susceptible check variety. The standard differentials, Albit, Hohenheimer, and Ridit, have been used in several other race classifications (10, 11, 13, 14); Orin and Omar have been used in 1 previous race study (10). Selections 50077 and 1403 are used here as new differential varieties.

The smut reactions of 4 of the 7 differential varieties used in this study (Table 1) are believed to be conditioned by single genes, while the reactions of each of the other 3 are determined by 2 or more genes. Albit, Selection 50077, Selection 1403, and Orin are single gene differentials, carrying, respectively, the M, M_2, H, and T or R genes (1, 2, 16). Omar carries the combined M and T factors (11). Hohenheimer and Ridit (3) have not been analyzed for specific resistance components and are therefore simply recognized as reaction types.

The seed of the differential varieties was rendered smut free and then inoculated by standard methods (13). Comparative tests were made at Pullman, Wash., and Pendleton, Ore., in 1958, 1959, and 1960. Smut percentages were determined on the basis of total heads of wheat in a 4-ft row.

EXPERIMENTAL RESULTS.—The data obtained (Table 1) showed that there are several race groups, each containing 2 or more races that condition a similar or identical reaction pattern. For example, every "L"

TABLE 1. Average percentages of bunt produced in 8 wheat varieties by 18 physiologic races of *Tilletia caries* (T-races) and 9 of *T. foetida* (L-races) at Pullman, Wash., and Pendleton, Ore., in 1958, 1959, and 1960

Race groups and races	Varieties, resistance factors, reactions,[a] and percentages of bunt							
	Hybrid 128 —	Albit M	Selection 50077 M_2	Selection 1403 H	Orin T or R	Hohenheimer Ho	Ridit rd	Omar TM
Martin								
	S	*S*	*R*	*R*	*R*	*R*	*R*	*R*
T-14	90	91	2	Tr[b]	0	0	1	0
	S	*R*	*S*	*R*	*R*	*R*	*R*	*R*
T-1	86	1	83	1	0	Tr	Tr	0
L-1	88	Tr	91	1	1	Tr	1	0
L-2	90	1	89	1	0	0	Tr	0
	S	*I*	*S*	*R*	*R*	*R*	*R*	*R*
T-2	89	28	76	2	0	0	1	0
	S	*S*	*S*	*R*	*R*	*R*	*R*	*R*
T-4	88	81	87	Tr	0	0	1	0
T-6	88	81	85	Tr	0	Tr	1	0
L-4	92	88	91	1	0	0	1	0
Hussar								
	S	*R*	*S*	*S*	*R*	*R*	*R*	*R*
T-3	94	Tr	88	87	0	0	6	0
L-3	90	Tr	88	90	0	0	4	0
	S	*S*	*S*	*S*	*R*	*R*	*R*	*R*
T-5	90	85	84	70	0	0	2	0
T-7	90	83	82	82	0	0	4	0
T-8	92	86	85	80	Tr	0	4	0
L-5	89	91	92	76	0	0	4	0
L-6[c]	—	—	—	—	—	—	—	—
L-7	91	90	89	88	Tr	0	6	0
Turkey								
	S	*R*	*S*	*R*	*S*	*R*	*R*	*R*
T-17	78	1	76	0	88	0	0	0
	S	*R*	*S*	*S*	*S*	*R*	*R*	*R*
L-8	88	Tr	84	89	87	0	1	0
	S	*R*	*S*	*S*	*S*	*S*	*R*	*R*
T-16	93	Tr	87	82	92	41	1	0
Hohenheimer								
	S	*R*	*R*	*R*	*R*	*S*	*R*	*R*
T-10	86	1	Tr	4	Tr	77	2	0
	S	*R*	*S*	*R*	*R*	*S*	*R*	*R*
T-9	90	1	72	2	0	65	0	0
	S	*S*	*S*	*R*	*R*	*S*	*R*	*R*
T-12	90	41	82	2	0	62	Tr	0
	S	*S*	*S*	*S*	*R*	*S*	*R*	*R*
T-15	90	86	88	86	0	82	2	0
Ridit								
	S	*R*	*R*	*S*	*R*	*R*	*S*	*R*
T-11	82	5	Tr	70	0	0	62	0
	S	*R*	*S*	*S*	*R*	*R*	*I*	*R*
L-10	84	2	88	90	0	0	20	0
	S	*S*	*R*	*S*	*R*	*R*	*S*	*R*
T-13	88	68	Tr	83	0	0	70	0
L-9	89	80	Tr	92	0	0	70	0
Omar								
	S	*S*	*S*	*R*	*S*	*R*	*R*	*I*
T-18	63	50	56	1	70	0	Tr	24

[a] R = Resistant (0-10% infection). I = Intermediate (11-40% infection). S = Susceptible (41-100% infection).
[b] Tr = Trace or less than 1%.
[c] Not available for testing.

(*T. foetida*) race, except L-8 and L-10, has a pathogenic counterpart among the "T" (*T. caries*) races. Thus, identical pairs are T-1 and L-1, T-4 and L-4, T-3 and L-3, T-5 and L-5, and T-13 and L-9. Furthermore, certain races within each species proved to be identical in the host testers. Identical pairs or groups within species are L-1 and L-2; T-4 and T-6; T-5, T-7, and T-8; and L-5, L-6, and L-7. Some of these apparent duplications are due to the fact that the spring wheat varieties on which the races were differentiated originally (13) were excluded from this study. The smut reactions of the spring wheat differentials, which primarily served to screen out border-line differences in pathogenicity among races otherwise identical on the winter wheat differentials, have generally been erratic and unreliable from year to year. For example, L-1

and L-2, which are identical on the winter testers, are differentiated by intermediate and susceptible reactions, respectively, of the spring wheat Marquis.

Further, the 1st reported differential reaction (13) proved to be incomplete in some cases, possibly because of a heterozygous condition in the original spore collection. Thus, originally T-4 was differentiated from T-6 and L-4 on the basis of an intermediate (T-4) and a susceptible (T-6 and L-4) reaction of Albit. Over the past 6 years, however, the stock spore collection originally identified as race T-4 has consistently conditioned a susceptible reaction of Albit, thus exhibiting the pathogenic pattern of T-6 and L-4 (Table 1). Likewise, T-7 was originally differentiated from races T-8 and L-7 by the intermediate *vs.* susceptible reaction of Hussar. In recent years of testing, however, the stock collections of races T-7, T-8, and L-7 have been indistinguishable. While race L-6 was not available for testing, it has been classed with the Hussar group (Table 1) on the basis of its earlier behavior, which was identical to T-7 (10, 13). Also, races T-5 and L-5, which originally were identified by the susceptibility of Martin and the resistance of Hussar (14), were later reported (2) to be pathogenic in Hussar as well, a report confirmed by this study.

The conclusion is suggested, therefore, that race grouping based on pathogenicity without regard for species category would be a more practical approach to servicing the program of breeding bunt-resistant varieties. Thus, the 28 currently recognized races have been reclassified into 17 pathogenic types on the basis of the reactions of the 7 differential varieties used in this study (Table 1). These 17 pathogenic types are further classified into 6 groups on the basis of their pathogenicity against 1 of the 6 generally recognized resistance types (Martin, Hussar, Turkey, Hohenheimer, Ridit, and Omar).

The Martin group consists of 4 pathogenic types characterized by their pathogenicity against either or both Martin factors (M and M_2) occurring alone. Race T-14 of the Martin group attacks varieties carrying only the M gene for resistance. Race T-1, L-1, and L-2 are pathogenic in those varieties whose resistance is conditioned by the M_2 gene alone. On the original set of winter tester varieties (14), these 3 races infected only the susceptible check, Hybrid 128, indicating that all known resistance factors and types controlled them. With the addition of a tester variety carrying the isolated M_2 gene (Selection 50077), these races are shown to be pathogenic in varieties carrying this single resistance factor.

Race T-2 of the Martin group conditions an intermediate and susceptible reaction of varieties carrying the M and M_2 factor, respectively. Originally, T-2 behaved like T-1, L-1, and L-2 by smutting only varieties whose resistance was conditioned by the M_2 gene. For the past 5 years, however, the stock spore collection of race T-2 has tended to infect Albit and White Odessa, both of which carry the M gene alone. The fact that T-2 was originally described as a "durum" race is substantiated by its recognition here as a distinct pathogenic type.

Races T-4, T-6, and L-4 of the Martin group are characteristically pathogenic in varieties carrying both Martin factors (M and M_2).

The Hussar group of races consists of 2 pathogenic types; both are pathogenic in varieties whose resistance is conditioned by the Hussar (H) gene (Table 1), but they are differentiated by the M gene (Albit).

Races T-3 and L-3 of the Hussar group, which appeared to be identical with T-1, L-1, and L-2 under the 1945 classification (14), are distinct from these races in the new testers. They infected Selection 1403 (H) in addition to Selection 50077 (M_2) (Table 1). In the testers used formerly, this reaction was masked by the presence of the M gene in the variety Hussar (1).

Six races (T-5, T-7, T-8, L-5, L-6, and L-7) constitute the 2nd pathogenic type of the Hussar group. These differ from T-3 and L-3 by the susceptibility of that Martin resistance conditioned by the M gene.

Three pathogenic types, each represented by a single race, comprise the Turkey group of races (Table 1). All attack varieties whose resistance is conditioned by the Turkey (T) or Rio (R) genes are differentiated by the presence of the H gene, and the Hohenheimer-type resistance. Races T-17 and L-8, pathogenically identical in the original testers (11), proved to be different in the new testers by the differential reaction of Selection 1403 (H). Race T-16 of the Turkey group differs from L-8 only in its ability to attack Hohenheimer.

The Hohenheimer group is composed of 4 pathogenic types (Table 1) which commonly attack Hohenheimer and are differentiated by the Martin and Hussar genes. In the original set of testers (13), T-9 and T-10 were differentiated in Hohenheimer by intermediate and susceptible reactions, respectively. This differential reaction gradually disappeared; only the addition of the differential Selection 50077 (M_2) makes possible their continuance as distinct races.

Race T-12 of the Hohenheimer group is characterized by its pathogenicity in Albit (M) and Selection 50077 (M_2), in addition to Hohenheimer; thus, T-12 is distinguished from T-15 of this group which attacks all of these differentials, as well as Selection 1403.

The 3 pathogenic types of the Ridit group are differentiated by the M and M_2 genes (Table 1). The M factor (Albit) conditions resistance to T-11 and L-10, but not to T-13 and L-9, whereas the M_2 factor (Selection 50077) conditions resistance to all but L-10 of the Ridit races. Races T-11 and L-10 of the Ridit group were originally differentiated by the Ridit susceptible and intermediate reactions, respectively. The susceptibility of Selection 1403 (H) is an additional differential reaction for both races, while the susceptibility of Selection 50077 (M_2) to L-10 makes possible a more positive differentiation of this race from T-11.

Only T-18 comprises the Omar race group, which is characterized by pathogenicity in Omar (Table 1), a

variety that combines the Turkey and Martin resistance factors. It was collected from Omar (11) and is the only race of common bunt known that can infect a variety carrying both the Martin and Turkey types of resistance. These resistance sources have been used most commonly in breeding bunt resistant varieties.

DISCUSSION.—In the Pacific Northwest, pathogenic races of *T. caries* and *T. foetida* are pertinent primarily to the problem of smut control in winter wheat production. Hence, the identity and stability of races in a standard system representing all known pathogenic types are essential to a study of the genetics of smut resistance and to a systematic program of breeding improved, smut-resistant varieties. This proposed system of race classification is designed to facilitate all studies of smut resistance. Specific race identity will aid in elucidating the genetic aspects of smut resistance, whereas race grouping by resistance type aids in screening large numbers of varieties for new sources of resistance.

Duplication of pathogenic types occurring in both species has been eliminated. Likewise, races differing only slightly in their pathogenic patterns have been grouped. Consequently, the present classification of 28 pathogenic types has been reduced to 17, representing 6 race groups. The original race numbers are retained to avoid possible confusion that might develop from assigning new numbers to races of long standing identity.

Organizing races into 6 groups, based on resistance type, is intended to facilitate the association of related fungus pathogenicity and host resistance. This poses a problem for races like T-16, which might be assigned to either the Hohenheimer or Turkey group. These races were assigned to the latter group because the Turkey type resistance has been of major importance in the breeding program whereas Hohenheimer is useful primarily for race differentiation. For varietal breeding purposes, therefore, races like T-16 are more closely allied with the Turkey group.

The practicality of this race grouping is substantiated by the fact that many varieties and hybrid selections have been successfully screened for resistance to common bunt in the past 5 years, largely on the basis of these 17 recognized pathogenic types (Table 1). In some cases, successful screening of varietal material has been accomplished by using but a single race from each of the 6 race groups. This race classification system, therefore, makes possible the testing of a large number of varieties in 1 season, since adequate screening is provided by a reduced number of races.

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PROPERTIES AND SEROLOGY OF PURIFIED CAULIFLOWER MOSAIC VIRUS

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SUMMARY

Tendergreen mustard (*Brassica perviridis*) was a good source of cauliflower mosaic virus for purification studies since a high titer of virus was present in systemically infected leaves. The virus was purified by homogenizing infected leaves in strong buffer, and clarifying the homogenate with n-butanol. This was followed by differential and density gradient centrifugation. The virus was found to be a spherical nucleoprotein 50 mμ in diameter, containing about 34% RNA, with a sedimentation constant of 222 S. Analytical ultracentrifugation and electrophoresis both indicated the presence of a single component. Specific antisera with titers of 1:1,280 were prepared by injecting rabbits with purified virus preparations.

INTRODUCTION.—Cauliflower mosaic virus is 1 of 2 viruses most commonly affecting crucifers in the United States. Reports of mechanically transmissible viruses of crucifers were made as early as 1921 (6, 10), but the 1st detailed account of the properties of this virus was given in 1937 (14). Subsequently, many reports have been made on its occurrence, and several strains have been described (2, 15, 16).

In general, the previously reported properties include both mechanical and aphid transmissibility, a thermal inactivation point of 70-75°C, a dilution end point of about 1:2,000, and resistance to aging *in vitro* for 7-14 days. The host range appears limited to the Cruciferae.

For many years, attempts have failed to determine the morphology of the virus and to prepare an antiserum to it (1, 7, 8). Recently, the authors published a brief account (9) of the purification of this virus and of the production of an antiserum. Almost simultaneously Day and Venables (3) published a similar report. Neither of these preliminary accounts contained much detail concerning the purification and characterization of the virus. The purpose of this paper, therefore, is to set forth in detail the modification of the procedure used by others (13) which led to the purification, and to present additional data on the physical-chemical properties of the virus.

MATERIALS AND METHODS.—The cabbage virus B strain, originally described by Walker, *et al.* (16), was used in this study. Virus cultures were maintained in cauliflower (*Brassica oleracea* v. *botrytis* L.) or tendergreen mustard (*B. perviridis* Bailey) kept in insect-proof cages.

Plants used as sources of virus for purification were inoculated at the 2-leaf stage, and harvested when systemic symptoms were fully developed. Powdered Carborundum was regularly used as an abrasive for all inoculations, and inoculum was applied with cheesecloth pads.

Purification was done in a cold room at 2-5°C. Leaves were homogenized for 4-5 minutes in a Waring Blendor. The initial low-speed centrifugation of extracted sap was carried out in a Servall model SP centrifuge. All subsequent centrifugation was done in a Spinco model L ultracentrifuge. Infectivity assays were made on tendergreen mustard plants at the 2-3-leaf stage.

Samples for electron microscopy were sprayed on collodion-coated grids, and air- or freeze-dried. The grids were shadowed with uranium and examined with an RCA type EMU2 electron microscope.

Two types of tests were used in making serological determinations. In the ring test, about 0.2-ml antiserum was placed in the bottom of glass tubes of 2-3 mm diameter. A similar volume of antigen was layered over the serum without mixing. After 10-20 minutes at room temperature, the tubes were examined under a bright light, and the amount of precipitate that had developed at the interface in the tubes was scored. Used in most determinations was the microprecipitin test described by Van Slogteren (17) since it was more sensitive and required less antigen and antiserum than the ring test.

Selection of host and assay plants.—Detailed tests with several commonly reported hosts of cauliflower mosaic virus, as well as with tendergreen mustard, showed that symptom expression occurred earlier, was more uniform, and lasted longer on tendergreen mustard than on the other hosts. The dilution end point of the virus was consistently higher in extracts from tendergreen mustard than from cabbage or cauliflower. The dilution end points for the virus extracted from the latter hosts ranged from 1:1,000 to 1:10,000 and are comparable with those previously reported for these virus-host combinations (2, 14, 16). Virus extracted from infected mustard leaves had dilution end points ranging from 1:10,000 to 1:100,000.

The relatively high titer of virus present in tendergreen mustard plants recommended the use of this plant as a virus source for purification studies. The decision to use it as an assay plant was based mainly on the rapidity and uniformity of its response to the

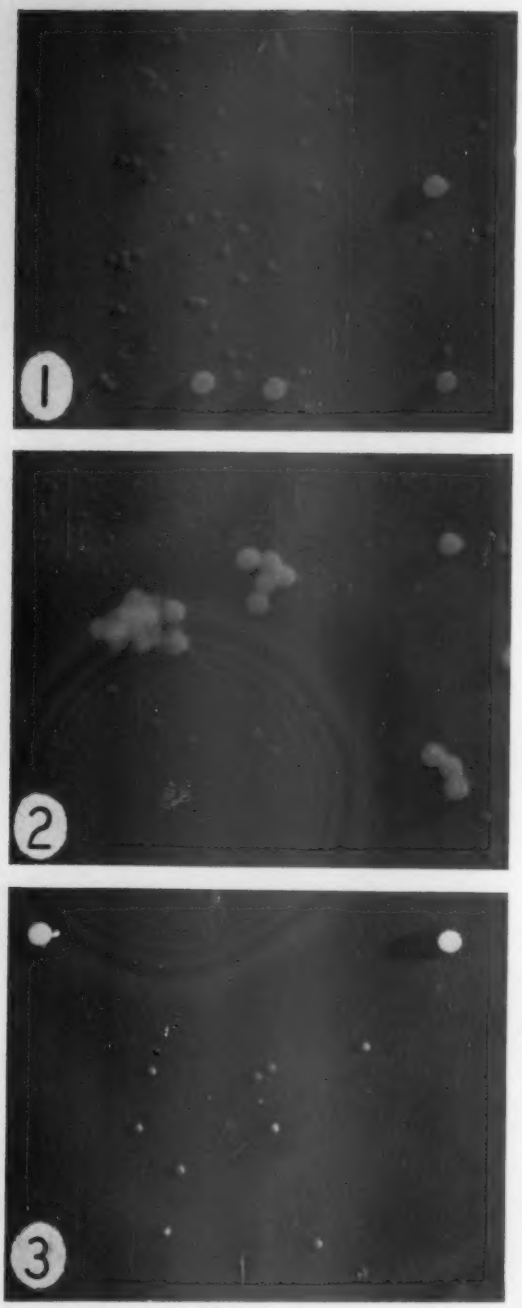

Fig. 1 3. 1) Electron micrograph of an air-dried preparation of cauliflower mosaic virus partially purified from tendergreen mustard. **2)** Electron micrograph of an air-dried preparation of material prepared from healthy tendergreen mustard leaves. **3)** Electron micrograph of a freeze-dried preparation of purified cauliflower mosaic virus. All figures × 24,500.

virus. In addition, reliable symptom expression occurs over a wide range of temperatures. Masking of virus symptoms at high temperatures has been shown to occur in cabbage and, to a lesser extent, in cauliflower. Summer temperatures sufficient to cause masking in both these hosts did not have a similar effect on tendergreen mustard, although symptoms appeared less severe in summer than in cooler months.

Purification of the virus.—Attempts to purify the virus by the ammonium sulfate method described by Takahashi (12) for the purification of radish mosaic virus, and by heat clarification followed by ultracentrifugation, as described by Martini (8), resulted in the loss of a great deal of virus infectivity as measured by the dilution end point method.

Preliminary experiments by the method used by Tomlinson, *et al.* (13) for purification of cucumber mosaic virus indicated that the yield of partially purified virus was greater with this procedure than with the other methods. Loss of virus, however, was still considerable. Stepwise modifications of this basic procedure were then undertaken in an attempt to increase the yield of infectious virus.

The modified procedure gave the best results with cauliflower mosaic virus (Fig. 5). Incubation of filtered homogenates with 7-8% butanol gave good clarification and formed compact pellets of host chloroplastic material in the tubes after centrifugation, instead of thick pellicles at the tops of tubes, when higher concentrations of butanol were used. This allowed greater ease of removal of the clarified liquid and yielded highly infectious supernatants after slow-speed centrifugation. Homogenates gave more highly infectious preparations after incubation with butanol for 1 hour than for longer periods.

The resuspension of sedimented virus pellets in 0.01 *M* phosphate buffers following high-speed centrifugation appeared to result in some loss of virus during subsequent low-speed centrifugation. Comparative tests showed that, after low-speed centrifugation, virus from pellets resuspended in water retained more infectivity than virus from pellets resuspended in buffers. This loss of virus may have been due to aggregation. In later experiments, examination of purified virus preparations in an analytical ultracentrifuge showed that a single schlieren peak occurred with virus suspended in distilled water. Similar preparations suspended in phosphate showed a major peak, corresponding to that observed in the water suspensions, as well as a faster-moving inhomogeneous component probably due to the presence of aggregated virus in the suspension. Comparative assays of crude sap extracts and final virus resuspensions made up to equivalent volumes indicated that loss of infectivity was slight when this purification procedure was followed.

Electron microscope examination of infected material after clarification and a single cycle of differential centrifugation revealed both large, somewhat flattened particles of about 60 mμ and smaller particles of 16-20 mμ (Fig. 1). This indicated that purification of the

virus by this procedure was not complete. Examination of preparations from healthy tendergreen mustard plants treated in a similar manner showed only the smaller particles (Fig. 2).

The relatively large difference in size between the virus particles and the particles in preparations from healthy plants indicated that separations could be achieved by differential centrifugation. A decrease in time of high-speed centrifugation to 1 hour at 30,000 rpm or ½ hour at 40,000 rpm resulted in complete sedimentation of the virus particles, with less contamination by host material.

Density gradient centrifugation was used to complete the removal of all host material. One ml of concentrated partially purified material was layered on 30-ml gradients of 5-40% sucrose in distilled water. Examination of tubes after centrifugation for 2 hours at 25,000 rpm in an SW-25 swinging bucket rotor, showed a distinct opalescent band midway down the tubes. No such band was present in tubes layered with similarly treated material from uninfected plants (Fig. 4). Tubes containing either virus-infected or healthy preparations showed 2 diffuse, barely discernible opalescent zones near the top of the tubes. Electron microscope examination of the material removed from the distinct band in the tubes containing the virus preparation, after removal of the sucrose by dialysis, revealed only the larger particles observed previously in the partially purified preparations. The virus removed from sucrose gradient tubes was infectious at dilutions of 10^{-5}. The material from the faintly visible bands at the top of the tubes was noninfectious.

Size and shape.—Examination of air-dried preparations of purified virus with the electron microscope showed that considerable flattening of the particles apparently had occurred. To make an accurate determination of the size and shape, purified virus was freeze-dried by spraying onto collodion-coated grids contained in specially designed heavy glass containers packed in dry ice. The containers were sealed while cold and the contents subjected to high vacuum. Specimens thus prepared did not appear flattened. They were spherical with a mean diameter of 50 mμ (Fig. 3). These observations agree quite closely with those reported by Day and Venables (3).

Sedimentation constant.—Purified virus samples were tested in a Spinco model E analytical ultracentrifuge in order to determine the sedimentation constant of the virus and to check the homogeneity of the preparations. Samples suspended in distilled water or buffer were run at 17,980 rpm. A single schlieren peak was observed during centrifugation of samples in distilled water at pH 5.9-6.2, indicating the presence of a single component (Fig. 6). The sedimentation constant of the virus under these conditions was 220-222 S at 20°C. Virus suspended in pH 6.0 phosphate buffer with an ionic strength of 0.2 also was found to have a major component with a sedimentation constant of 222 S, but a faster-moving component—probably aggregated virus—also was present.

Fig. 4. Right) Zone produced in a sucrose gradient tube layered with a partially purified virus preparation. Left) Tube layered with material from healthy plants.

The data of Day and Venables (3) also indicated a single component and a sedimentation constant of 214 S.

Ultraviolet absorption.—Fig. 7 shows the ultraviolet absorption spectrum of a purified preparation of cauliflower mosaic virus, as measured with a Carey recording spectrophotometer. The virus was suspended in water at pH 5.9. The curve is typical for nucleoprotein, with the absorption maxima and minima near 260 and 240 mμ, $E\frac{260}{280} = 1.39$ and $E\frac{max}{min} = 1.17$. This absorption spectrum was nearly identical to that reported by Day and Venables (3).

Electrophoretic analysis.—Purified virus preparations in KH_2PO_4-K_2HPO_4 buffer with an ionic strength of 0.12 showed a single peak upon analysis in the moving boundary electrophoresis apparatus (Perkin Elmer model 38A) at both pH 6.5 and 7.5, again indicating the presence of a single component in these preparations. Although the presence of aggregated virus in such systems was indicated by ultracentrifugal analysis, both aggregated and unaggregated virus appeared to migrate together in the electrophoresis apparatus. Electrophoretic analysis made by Day and Venables (3) indicated 3 components in their preparation.

Type and percentage of nucleic acid.—Purified virus preparations were hydrolyzed to release the purine-bound sugars by heating 1 hour at 100°C with an equal volume of 2*N* HCl. The presence of pentose was indicated by a strong positive reaction with orcinol, using the method described by Dische (4). The pres-

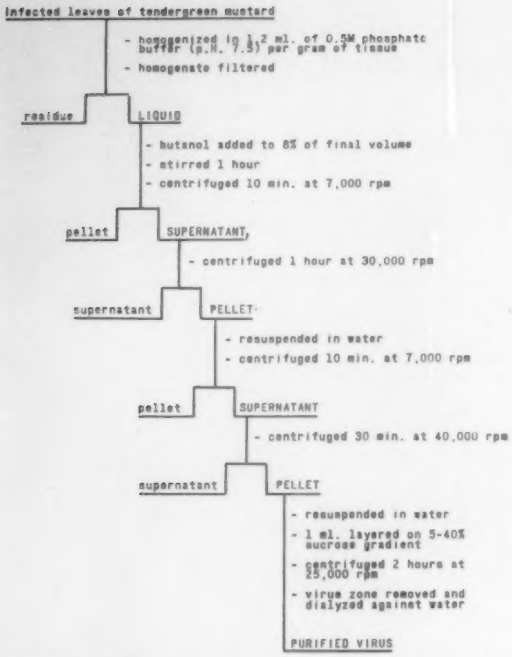
Infected leaves of tendergreen mustard
- homogenized in 1.2 ml. of 0.5M phosphate buffer (p.H. 7.5) per gram of tissue
- homogenate filtered
residue
LIQUID
- butanol added to 8% of final volume
- stirred 1 hour
- centrifuged 10 min. at 7,000 rpm
pellet
SUPERNATANT,
- centrifuged 1 hour at 30,000 rpm
supernatant
PELLET
- resuspended in water
- centrifuged 10 min. at 7,000 rpm
pellet
SUPERNATANT
- centrifuged 30 min. at 40,000 rpm
supernatant
PELLET
- resuspended in water
- 1 ml. layered on 5-40% sucrose gradient
- centrifuged 2 hours at 25,000 rpm
- virus zone removed and dialyzed against water
PURIFIED VIRUS

Fig. 5. Outline of the procedure used in the purification of cauliflower mosaic virus.

ence of ribose also was detected chromatographically. Hydrolyzed virus and ribonucleic acid preparations, as well as D-ribose standards, were spotted on Whatman No. 1 paper. Ascending chromatograms were run for 14 hours in a phenol-saturated, citrate-phosphate buffer system at pH 5.7. The chromatograms were developed by spraying with aniline hydrogen phthalate and heating 5 minutes at 105°C. Spots formed by ribose, hydrolyzed RNA, and hydrolyzed virus all had similar R_F values, indicating that the virus nucleic acid was of the ribose type.

The percentage of nucleic acid in the virus was determined by a modification of the optical method described by Englander and Epstein (5). The percentage concentration of nucleoprotein in purified virus preparations was determined by comparing the area under the ultracentrifuge pattern produced by the virus with that produced by a 1% solution of ribonuclease, used as a protein standard. These patterns were enlarged with a photographic enlarger, and the relative areas measured. The percentage concentration of virus nucleoprotein then was calculated by comparison with the standard.

A subsample of the virus preparation used to calculate nucleoprotein concentration was used to determine nucleic acid concentration. The optical density of the solution at 260 mμ was determined with a Carey recording spectrophotometer. This value was then corrected for light scattering and for absorption by proteins. The percentage concentration of nucleic acid was determined by comparison with the value obtained for a known concentration of ribonucleic acid from tobacco mosaic virus.

The percentage of nucleic acid in the virus was then calculated from the values obtained for the nucleoprotein and nucleic acid concentrations. In 2 determinations, this value was found to be 35.3 and 33.8% nucleic acid.

Serological studies.—In an initial experiment, 2 methods of preparing an antiserum to the virus were compared. One-ml portions of a concentrated partially purified virus preparation were injected into 2 rabbits. One rabbit was injected intravenously with the entire amount, whereas the other was given 0.5 ml intravenously. The other 0.5 ml was emulsified with an equal amount of Freund's incomplete adjuvant and injected intramuscularly. Injections were made at 10-14-day intervals, and test bleedings were made just before the next injection. The titers of the antisera were determined by testing against partially purified virus preparations, using ring tests. The titer of the antiserum prepared by giving both intramuscular and intravenous injections was consistently higher than that of the antiserum produced only by intravenous injection. After the 7th injection, both rabbits were given intramuscular injections, and within 2 weeks both rabbits yielded antiserum with a titer of 1:1,024.

Antisera produced in response to the injection of partially purified virus gave no apparent reaction when tested against comparable preparations from healthy plants, if ring tests were used. In later experiments in which the microprecipitin test was used, detectable precipitates occurred with partially purified extracts from healthy plants at antiserum dilutions up to 1:20.

In an attempt to produce a more specific antiserum, another rabbit was injected both intramuscularly and intravenously with purified virus preparations from sucrose gradients. The antiserum produced by this rabbit gave precipitates out to dilutions of 1:1,280, when tested against partially purified or purified virus preparations. Partially purified material from healthy plants did not react with this antiserum. Normal serum was used as a control in all of these experiments, and in no case did it react with any of the preparations tested.

Numerous attempts were made to use the antiserum for the detection of virus in clarified extracts, using the microprecipitin test. Sap extracts were clarified with butanol or by heating 10 minutes at 50°C. When butanol-clarified extracts from either healthy or virus-infected plants were tested, some nonspecific precipitation occurred with both antiserum and normal serum. This precipitate was not typical, however, of that observed when partially purified virus was tested against the antiserum. In addition, some precipitation also occurred when antisera prepared with partially purified virus were tested against healthy sap extracts.

Usually, when clarified extracts from virus-infected plants were tested, some of the typical precipitate was

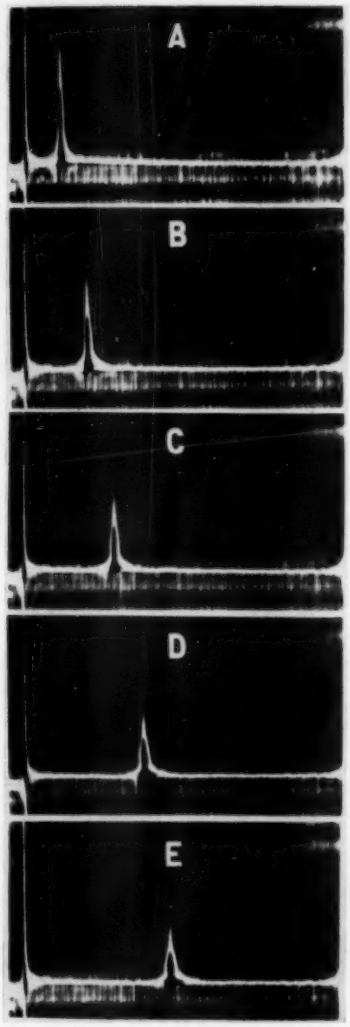

Fig. 6. Sedimentation pattern of a purified virus preparation centrifuged at 17,980 rpm. The pictures, A-E, were taken at 4-minute intervals.

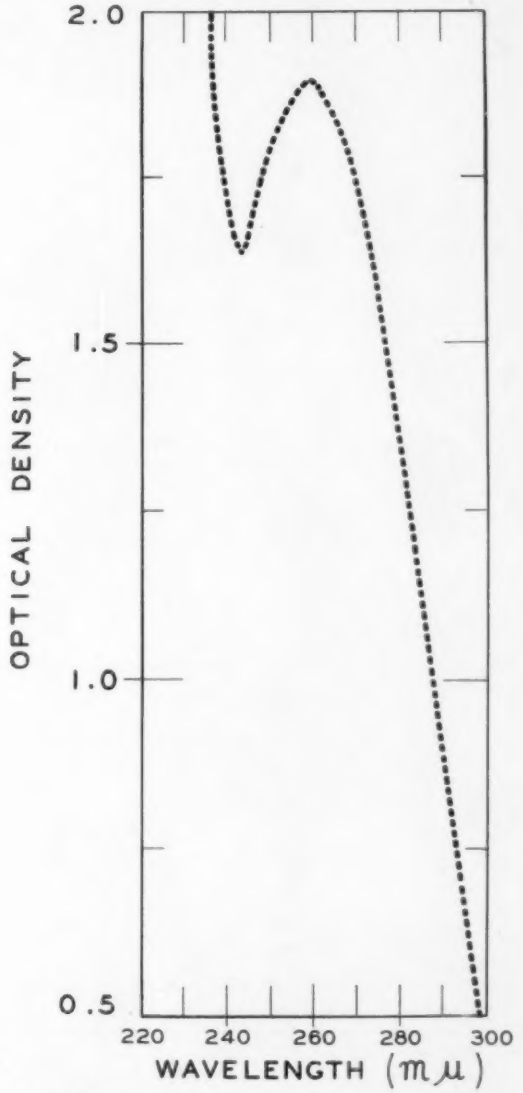

Fig. 7. Ultraviolet absorption spectrum of a purified cauliflower mosaic virus preparation.

formed. If the clarified sap was diluted more than 1:4, however, this precipitate usually could not be observed. In some cases, none of the typical precipitate could be observed at any dilution. No precipitation occurred when butanol-buffer controls were tested against the antisera. Sap extracts clarified by heating showed less of the nonspecific type of precipitation, but none of the typical virus precipitate was formed in any of the tests.

To test the possibility that the virus was often present in the plant in too low a concentration to be detected serologically, comparative tests were made against virus in butanol-clarified sap, and partially purified concentrated virus preparations. A sample of the clarified extract was partially purified by 1 cycle of high- and low-speed centrifugation. The partially purified virus, which had been concentrated to 0.05/ initial volume then was tested in a series of 2-fold dilutions against the antiserum. The virus gave definite precipitates up to a concentration equivalent to 0.5/ initial volume. At a concentration equal to that occurring in the clarified sap, only a faint precipitate occurred. Typical precipitates could not be observed with any of the dilutions of clarified sap, probably

due in part to masking by the nonspecific precipitation.

The results of the tests carried out so far with clarified sap indicate that the reaction is not specific or intense enough to be used to detect the presence of the virus in such extracts.

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SOWBANE MOSAIC CAUSED BY A SEED-TRANSMITTED VIRUS

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SUMMARY

Sowbane mosaic virus, isolated from a plant of *Chenopodium murale*, produced mottling on 14 species of chenopods, but failed to infect any of 112 other species of plants, representing 32 families. It produced local lesions on *Beta macrocarpa*, *B. vulgaris*, *Chenopodium quinoa*, *C. murale*, and *C. amaranticolor*; of these only *C. quinoa* and *C. murale* were invaded systemically. The virus was transmitted readily by juice inoculation and through seeds of *C. murale*, *C. album*, and *Atriplex pacifica*. Virus was recovered from pollen of *A. coulteri* but no evidence of seed transmission was found in this species. *Circulifer tenellus*, *Liriomyza langei*, *Halticus citri*, and probably *Myzus persicae*, are vectors. *C. tenellus* and *H. citri* were infective after 8 hours of fasting. They transmitted virus in each of 4 successive transfers at 2-hour intervals. No transmission was obtained from vectors ground and mixed with water. Juice from diseased plants mixed with macerated insects produced about 50% as much infection as juice alone. Ability of the vectors to transmit may be associated with failure of animal derivatives, such as saliva, to prevent infection. The virus has a thermal inactivation point between 84° and 86°C. It remained active *in vitro* 2 months, and withstood dilutions of 10^{-5}-10^{-8}. It retained activity in dried leaves for 2 years and was transmitted by seeds stored 6½ years.

Introduction.—In a survey of plants in beet fields near Riverside, Calif., in 1952, for beet yellows virus in weeds, a type of mottling was found on a plant of *Chenopodium murale* L. (sowbane or nettleleaf goosefoot) which was caused by a juice-transmissible virus. The virus has not been found again on plants growing under natural conditions. As the virus appeared to have some rather distinctive characteristics and to be undescribed, it was studied further and the results are reported here.

Since the virus was discovered in sowbane and causes marked symptoms on this plant, the induced disease is designated "sowbane mosaic" (SM) in conformity with usage adopted in The Review of Applied Mycology in applying common names to virus diseases (1).

Symptoms and host range.—The most conspicuous symptoms of sowbane mosaic are produced on *C. murale* and *C. quinoa* Willd. Local, circular, chlorotic lesions, which attained a diameter of about 2 mm, began to appear on inoculated leaves of these species 3-4 days after inoculation with juice from diseased plants. The lesions were easily counted on inoculated leaves of *C. quinoa*, but they were usually less conspicuous on leaves of *C. murale*. Five-seven days after inoculation, systemic infection was evident. Clearing of the veins of young leaves appeared first, followed by clearly defined mottling or yellowing. Some affected leaves showed numerous chlorotic spots and were normal in shape, others had large chlorotic areas, often more evident on the basal half of the leaf. Some leaves were yellow and malformed (Fig. 1-A). Infected plants were dwarfed.

Symptoms on *Spinacia oleracea* L. were similar to those on *C. murale*, but less severe. Other plants which became infected systemically and which showed mottling, include *Atriplex hortensis* L. (garden orach), *A. rosea* L. (redscale), *Chenopodium album* L. (lambsquarters), *C. capitatum* (L.) Aschers (strawberry-blite), *C. urbicum* L. (city goosefoot), *Kochia scoparia* (L.) Schrad. (summer cypress), and *Monolepis nuttalliana* (Schultes) Greene. Mottling was produced also in young plants of *Atriplex cordulata* Jepson, *A. coulteri* (Moq.) D., *A. expansa* S. Wats., and *A. pacifica* Nels., but these species tended to recover.

In addition to the 14 species in which systemic infection was produced, local lesions were obtained by juice inoculation to *Beta macrocarpa* Guss., *B. vulgaris* L. (sugar beet var. US 75), and *C. amaranticolor* Coste & Reyn. Local lesions on *B. macrocarpa* were circular, about 1 mm in diameter, and soon became necrotic (Fig. 1-B). Lesions on sugar beet were circular and so small that they were barely visible as sunken necrotic spots. Lesions on *C. amaranticolor* were larger, attaining a diameter of 2-3 mm. They did not become necrotic.

In host-range studies, made in connection with similar studies of sugar beet yellows virus, 112 other species, representing 32 families, were tested, but none showed evidence of infection. All species infected belong to the family Chenopodiaceae, indicating a very restricted potential host range.

Transmission.—As has been indicated already, SMV is transmissible by juice inoculation. No transmission was obtained by means of *Cuscuta californica* Choisy and no infection was obtained with juice of dodder growing on infected plants of *Chenopodium murale*, indicating that this species of dodder may be immune from infection.

Transmission by vectors.—The virus which causes sowbane mosaic was shown by Costa, *et al.* (3), to be transmissible by means of adults of the leaf-miner fly, *Liriomyza langei* Frick. Percentage of infection was relatively low and apparently transmission was mechanical by virus carried on the mouthparts, ovipositor, or both. This evidence suggested that perhaps other insects with mouthparts which penetrate or abrade plant tissues may also act as vectors of this virus. To obtain information on potential vectors, further tests were made with *L. langei* along with tests with *Circulifer tenellus* (Baker) (beet leafhopper), *Halticus citri* Ashmead (fleahopper), and *Myzus persicae* (Sulz.) (green peach aphid).

Since SMV is easily transmitted mechanically, precautions were taken to avoid accidental infection in vector tests. *Circulifer tenellus* and *H. citri* were allowed to feed 24 hours on infected plants of *Chenopodium murale* and then transferred to healthy seedling plants of *C. murale* by means of a pipette. The leafhoppers and fleahoppers were removed after 4-6 hours and the plants were incubated in a screened compartment. The plants were inoculated in groups of 6, and 60 leafhoppers or 60 fleahoppers had access to each group of plants. The inoculations were made in cages which fitted over the groups of plants without coming in contact with them and the plants were not touched with any instruments during the course of feeding or removal of the leafhoppers or fleahoppers. Check plants were used on which leafhoppers and fleahoppers were fed on healthy plants of *C. murale*. In tests with *M. persicae*, aphids were placed on infected plants in a screened compartment and shaken onto healthy plants at intervals. An average of at least 100 aphids had access to each healthy plant over a period of 10 days. Tests with *Liriomyza langei*, made for comparison, were conducted as indicated for *C. tenellus* and *H. citri*.

Transmission through seeds.—Seeds were harvested from infected plants of *Chenopodium murale* and tested in the greenhouse for transmission of the virus. Many subsequent tests were made in which seed transmission in this species ranged from 20-70%. Symptoms often were marked on the cotyledons even before they reached maturity. Tests were made with other species of plants infected systemically and which showed identifiable symptoms. The results of these tests (Table 1) show that the virus was transmitted through seeds of *Atriplex pacifica*, *C. album*, and *C. murale*, but not through seeds of 7 other species.

Some of the species tested for seed transmission showed a tendency toward recovery. Since seedlings from such plants might also show less evident symptoms than seedlings inoculated directly, tests were made to determine whether virus was present in seedlings of all species in which there was no evidence of seed transmission. In these tests, juice was pressed from lots of 25-50 seedlings and inoculated into leaves of *Beta macrocarpa*. No indication of virus was found in any of the seedlings from seeds of the species which showed no symptoms. Further tests were made in which seedlings from infected plants were transplanted to pots and inoculated with SMV. In all instances, plants became infected and showed symptoms. It seems probable, therefore, that the virus was

TABLE 1. Seed transmission of sowbane mosaic virus

Plant tested	No. of seeds planted	No. of seedlings obtained	% seed transmission
Atriplex cordulata	200	115	0.0
A. coulteri	200	95	0.0
A. expansa	200	91	0.0
A. hortensis	1270	820	0.0
A. pacifica	300	98	21.4
A. rosea	1650	1027	0.0
Chenopodium album	600	303	30.0
C. murale	775	327	45.6
Monolepis nuttalliana	500	85	0.0
Spinacia oleracea	506	251	0.0

not transmitted through seeds of 7 of the 10 species tested.

Since SMV is readily transmissible by mechanical inoculation, tests were made with seeds of *C. murale* to determine whether the virus in seedlings was carried within or on the surface of the seeds. In the series of tests, seeds from infected plants were washed in a 10% solution of Na_3PO_4, to remove virus from the surface, and then planted singly in flats. Transmission through washed seeds was not significantly different from that through unwashed seeds of the same lot.

Tests also were made in which seeds from healthy plants were immersed in juice from infected plants and then dried and planted in flats. No infected seedlings were obtained in these tests. Probably, therefore, the virus involved in seed transmission of SMV is carried within the seed; virus carried on the surface of the seed probably would not produce infected seedlings under the test conditions.

Properties of the virus.—Silva, *et al.* (5), in studies of sowbane mosaic virus supplied by the junior author, succeeded in purifying and photographing the virus. They found that the virus particles are spherical and about 30 mμ in diameter.

Other studies of properties of the virus were conducted at the U. S. Agricultural Research Station, Salinas, Calif., where work was done with juice extracted from diseased leaves of *C. murale*. After treatment, the inoculum was introduced into leaves of *B. macrocarpa* by the rubbing method of inoculation with an abrasive and results were recorded as local-lesion counts.

Thermal inactivation.—Juice from infected plants was placed in small, thin-walled test tubes and subjected to the desired temperature for 10 minutes. All leaves of individual test plants were inoculated and local lesions on the 3 leaves showing largest numbers of lesions were counted. The virus titer was reduced at 75°C and decreased progressively until all virus was inactivated at 86°C. The thermal inactivation point of the virus appears to lie between 84° and 86°C.

Longevity in vitro.—Tests, in which juice from diseased plants in flasks or tubes was held at room temperature for different periods before being used as inoculum, gave a wide range of results. In some instances, the virus was inactivated in 2-4 weeks; in others, the virus survived 2 months. These results indicate a relatively high degree of resistance to inactivation in expressed juice.

Tolerance of dilution.—Juice was pressed from diseased plants of *C. murale* and diluted with distilled water. The various preparations then were inoculated into leaves of *B. macrocarpa* and *C. quinoa*. Results with *B. macrocarpa* indicated a tolerance of a dilution of 10^{-5} and results of repeated tests by the junior author with *C. quinoa* in Brazil indicated the unusually high tolerance of 10^{-8}. These results indicate a very high concentration of virus in infected plants.

Other properties.—The virus was active after 2 years in frozen juice of *C. murale*. Apparently, concentration was unimpaired and it seems likely that the virus will remain active indefinitely in a frozen medium. Tests at intervals indicated that the virus remained active for more than 2 years in dried leaves of *C. murale* stored in paper bags in the laboratory. There was indication, however, that concentration of virus in the leaves had dropped to a low level by the end of the 2nd year.

The virus has a long period of survival in stored seeds. Tests at irregular intervals of seeds of *C. murale* harvested February 18, 1953, showed that the virus was active after 6½ years of storage.

Relation of virus to vectors.—Tests were made in which 10 fleahoppers and 10 of each of the 2 species of insects tested as vectors were ground in a mortar and mixed with 1 ml of water. The mixtures were inoculated into leaves of *B. macrocarpa*. No transmission was obtained in these tests.

Failure of this inoculum to produce infection does not, of course, prove that the virus was not present in the inoculum, since there is considerable evidence that the presence of macerated insects in inoculum may prevent infection. Black (2) found that juices of several insect vectors, mixed in inoculum, greatly reduced or prevented infection with TMV and certain other viruses. Tests were made to determine whether macerated vectors have a similar effect on infection with SMV. Leafhoppers and fleahoppers were ground and mixed with juice from *Chenopodium murale* in the proportion of 10 individuals to 1 ml of a 1:100 dilution of juice. The mixture then was inoculated into leaves of *B. macrocarpa* and local-lesion counts were made on 2 cm^2 of leaf surface of each of 3 leaves. In 4 tests, juice mixed with *Circulifer tenellus* produced an average of 14.2 lesions compared with 25.1 lesions by juice alone. In 4 tests, juice mixed with *H. citri* produced an average of 10.6 lesions compared with 30.5 lesions with juice alone. The macerated vectors in the proportions used obviously did not prevent infection although they reduced it appreciably.

Further tests were made with *C. tenellus* and *H. citri* to determine the effect of fasting and of successive transfers on the time of virus retention.

Fig. 1. **A)** Leaves of *Chenopodium murale*, 1 healthy and 2 showing severe symptoms of sowbane mosaic. **B)** Leaf of *Beta macrocarpa* showing local lesions induced following mechanical inoculation with juice from *C. murale*.

In the fasting tests, vectors were removed from diseased plants of *C. murale* and placed in an empty cage at room temperature. At 2-hour intervals, 50 individuals of the species being tested were transferred to a group of 5 plants of *C. murale*. Infection was obtained with each species after all fasting periods including 8 hours, indicating that the virus is retained by each vector for at least 8 hours.

In the successive-transfer tests, 50 individuals of each species were transferred at 2-hour intervals on groups of 5 seedling plants of *C. murale*. Transmission was obtained on plants of all transfers indicating that the virus was retained by each species through 4 successive feeding periods on healthy plants. Infection percentage, however, was less in each successive transfer and very little infection was obtained with either vector in the 4th feeding period.

Discussion.—Sowbane mosaic virus appears to be a previously undescribed virus of unknown origin and of limited distribution. Certain of its characteristics appear to favor a more widespread occurrence. It is seed transmitted and readily transmitted mechanically; and 2 host plants, *C. album* and *C. murale*, are common weeds in cultivated areas throughout the United States and other parts of the world. The virus, however, has been found only once in 6 years under natural conditions. In addition to the 3 insects and the fleahopper shown to be able to transmit the virus, it is probable that other insects or agents may be capable of transmitting it under certain conditions. It seems unlikely, however, that any of the vectors are highly efficient in transmission. This vector relationship and the restricted host range probably account for the limited occurrence of the virus.

Although SMV probably will not become of appreciable economic importance, the virus is of interest because of certain of its properties and characteristics. With its high concentration in infected plants, its retention of activity at relatively high temperatures, and its ability to withstand desiccation and other adverse environmental conditions, it should be of value for use in basic studies of plant viruses.

Transmission of SMV by *C. tenellus*, *H. citri*, *L. langei*, and *M. persicae* also is of interest in relation to the factors that may be involved. There is considerable evidence that vectors which transmit mosaic viruses do so largely by introducing virus into living cells as a contaminant on their mouthparts. This is indicated by the short feeding periods needed to acquire and transmit certain mosaic viruses, and by the readiness with which such viruses are lost in successive feedings. If transmission were wholly mechanical, it seems likely that most any insect, or other biological agent that punctures or rasps plant tissue, would be able to transmit these viruses. It is a striking fact, however, that there is a considerable degree of specificity among insects with respect to transmission of mosaic viruses; and tobacco mosaic virus, which is most easily transmitted by juice inoculation, appears to be transmitted by insects rarely, or not at all, depending on the source plant, host plant, and possibly other factors (4).

The reason for specificity in the transmission of mosaic viruses by aphids and other biological agents which injure plant tissue in feeding is not known. It is possible, however, that transmission may be influenced to some degree by the types of materials introduced into the injured plant cells along with virus. Black (2) showed that small amounts of insect juice may prevent or greatly reduce infection with

TMV and certain other viruses. Many sucking insects deposit salivary secretions in the cells through which the mouthparts pass or in which the insects feed. Material of this kind may have a marked effect, and perhaps a differential one, on increase of viruses introduced into injured cells by biological agents. At any rate, the 2 viruses, SMV and TMV, appear similar in concentration in the plant, ease of mechanical transmission, and a number of other properties; but infection is difficult to obtain with TMV in the presence of insect juices, and with insects; whereas SMV produces infection in the presence of insect juices and is transmissible by such diverse agents as *C. tenellus*, *H. citri*, *L. langei*, and *M. persicae*.

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A MUTATION FOR RESISTANCE TO POTATO VIRUS Y IN PEPPER

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SUMMARY

A single plant, herein designated YRP10, of a popular commercial pepper variety susceptible to potato virus Y, was not infected, following mechanical or graft inoculation with a typical strain of the virus. Resistance to this virus (strain) was effected by a single recessive gene in the homozygous condition. It was concluded, from failure to demonstrate susceptible (recombinant) genotypes in progenies of YRP10 crossed with the resistant P11 variety, that the respective alleles for resistance to this virus strain were identical in both varieties. Susceptibility to tobacco etch virus and resistance to potato virus Y of YRP10 progeny plants constituted the 1st instance of this combination of responses to these 2 viruses. Therefore, the gene symbol ey^a must be considered invalid.

Introduction.—The prevalence and severity of potato virus Y (PVY) in field-grown bell peppers (*Capsicum annuum* L.) in Florida has been assessed to be of major importance (1, 8, 9). Two varietal sources of resistance to PVY have been reported and studied (2, 4). Inheritance studies of resistance to PVY and to tobacco etch virus (TEV) from these varieties demonstrated that the recessive genetic factors which conferred resistance to strains of these 2 viruses in both varieties could not be distinguished, hence the gene designation ey^a (5). An attempt has been made to incorporate resistance to these diseases into commercially acceptable plant types(3).

While studying the reactions of several commercial varieties to 1 strain of PVY, a single plant of a susceptible variety did not exhibit symptoms. This plant was designated YRP10. Presumably, the resistance of YRP10 was the result of a mutation. Because of the immediate need for resistant varieties, and because YRP10 possessed several other desirable characters, it was investigated further.

Materials and Methods.—The virus strains previously designated PVY-N, TMV-A, and TEV-E (2) were used throughout these experiments.

The resistant YRP10 was crossed with plants of the susceptible varieties California Wonder (CW), Florida Giant (FG), and Ruby Giant (RG), and the resistant variety P11 (2). Plants of the F_1 generation were back-crossed to both parental types.

Inocula were prepared from recently infected *Nicotiana tabacum* L. var. Turkish. Each test lot consisted of the entire plant population derived from the seed of a single pollination (*i.e.*, fruit). Plants of test progenies were dusted lightly with Carborundum before mechanical inoculation by the gauze-pad technique. Those plants which had not exhibited symptoms within 21 days after mechanical inoculation were reinoculated in the same manner and observed for an additional 3 weeks. The susceptible portion of grafted plants was not inoculated until at least 3 days after grafting. A small number of plants of the indicator variety Tabasco (*C. frutescens* L.) were included in all

inoculations as a check on virus identity and infectivity.

All plants were grown in screened greenhouses to prevent promiscuous pollination and infection of test plants by viruliferous insects.

RESULTS.—*Reaction to virus strains.*—The original plant, YRP10, was mechanically inoculated 6 times with PVY-N within the 1st 8 months following its discovery. During this period there were no symptoms indicative of infection. This plant, 24 months later, still has excellent vigor, and shows no indication of infection with PVY-N. Unsuccessful attempts at virus recovery were made from this plant as late as 113 days after the 1st inoculation.

Graft inoculation attempts were performed by grafting 3 scions from YRP10 on healthy CW stocks, and then inoculating the stocks mechanically with PVY-N (Table 1). No symptoms appeared in the scions within 212 days after inoculation of the stock portions. Symptoms were noted and virus was readily recovered from the stocks within 21 days after inoculation. All attempts to recover virus from these scions up to 113 days after inoculation were unsuccessful.

Comparable results were obtained using plants derived from the seed of selfed fruit of YRP10 (YRP10+1) and inoculated by both procedures mentioned. Seedlings of selfed YRP10+1 plants (YRP 10+2), inoculated as before, confirmed previous observations. In addition to the graft inoculation procedure outlined, reciprocal grafts were made in which healthy CW scions or YRP10-progeny stocks under healthy CW scions were inoculated. In the latter case, infection of the uninoculated CW scion would have indicated susceptibility of the YRP10 progeny stock, irrespective of symptom expression in the stock. But, as with the YRP10 plant and scions from it, it was not possible to infect plants of either progeny designation by mechanical or graft inoculation with PVY-N.

It had been concluded from previous studies with 2 other pepper varieties (P11 and S.C. 46252), resistant to strains of PVY and TEV, that resistance to these diseases in both varieties resulted from the pleiotropic effects of the same single recessive gene. To determine if this situation occurred in the mutant YRP10, a single YRP10+1 plant was mechanically inoculated with TEV-E. The uniform susceptibility of YRP10 progeny to this virus strain was confirmed when similar results were obtained with a larger sample of YRP10+2 plants.

The imperfect localization reaction (7) to TMV-A of the parent variety from which YRP10 was selected had been previously verified (unpublished). YRP10+1 plants exhibited a similar resistance to this virus strain (Table 1).

Inheritance of resistance to PVY-N.—The inheritance of resistance from YRP10 to PVY-N was determined by mechanical inoculation of F_1, back-cross, and F_2 progenies of YRP10 out-crossed with 3 susceptible varieties—CW, FG, and RG. The results of these inoculations are summarized in Table 2. Since

TABLE 1. Results from inoculation of YRP10 plants with strains of 3 viruses

Virus strain	Inoculation technique	Progeny	Days observed[b]	No. of test plants inf./inoc.[c]	Virus recovery
TEV-E	Mechanical	YRP10+2	14-31	29/29	.....
TMV-A	Mechanical[a]	YRP10+1	10-29	22[d]/22	.....
PVY-N	Mechanical	YRP10+1	54*-116	0/76	0/40[h]
	Mechanical	YRP10+2	56*	0/80	0/80
	Graft	YRP10	113*-212	0/3[e]	0/3
	Graft	YRP10+1	93	0/11[f]	.....
	Graft	YRP10+1	101*	0/24[g]	0/24
	Graft	YRP10+2	101*	0/20[g]	0/20
	Graft	YRP10+2	86*	0/20[g]	0/20

[a] Inoculum diluted 1:10,000.

[b] Number of days plants observed after inoculation. Asterisks indicate days between inoculation and attempted recovery of virus.

[c] Number of plants with visible symptoms/number of plants inoculated.

[d] Indistinct localization ($l^i l^i$) reaction.

[e] YRP10 scions grafted on California Wonder stocks (stocks inoculated).

[f] California Wonder scions grafted on YRP10+1 stocks (stocks inoculated).

[g] California Wonder scions grafted on YRP10+2 stocks (scions inoculated).

[h] Number of positive recoveries/number of recovery attempts. All recovery attempts made from uninoculated portions of grafted plants.

the P values derived from the χ^2 analyses were not significant, deviation from the proposed segregation ratios appeared negligible. Resistance to PVY-N in YRP10 and related progenies, therefore, was considered to result from a single recessive gene pair.

Resistance of YRP10 vs. P11.—Because of similar resistance to PVY-N in the varieties YRP10 and P11, the relation of genetic factors effecting resistance in each variety was investigated. Progenies inoculated for this purpose included the F_1, back-crosses to each parent, and F_2 of YRP10 crossed with P11. Table 3 shows only 2 susceptible plants of more than 7,000 plants inoculated. Virus was recovered from these 2 plants. Since resistance in both varieties was known to be effected by a single recessive gene pair, a chance out-cross by a single pollen grain from a susceptible plant would produce a heterozygous, but susceptible, plant. Although precautionary measures were taken against insect infestations, susceptible plants were on hand during the course of these studies and ants could have effected such out-crossings. Selfed fruit was harvested from both susceptible test plants. The progenies from these plants when inoculated did segregate in the manner expected; *i.e.*, 3 susceptible, 1 resistant, if derived from parent plants heterozygous for the allelic pair in question. From these studies, the recessive alleles in YRP10 and P11, that effect resistance when in the homozygous condition, were considered identical.

TABLE 2. Results from inoculation with PVY-N of progenies of YRP10 crossed with 3 susceptible varieties

Progeny	No. of plants Sus.	No. of plants Res.	Seg. ratio	P values for Total χ^2	P values for Pooled χ^2	P values for Inter. χ^2
California Wonder						
F_1	27	0	1:0	.99	.99	.....
BC(CW)	503	0	1:0	.99	.99	.99
BC(YRP10)	226	238	1:1	.3-.5	.5-.7	.2-.3
F_2	662	210	3:1	.9-.95	.5-.7	.8-.9
Florida Giant						
F_1	50	0	1:0	.99	.99	.....
BC(CW)	190	0	1:0	.99	.99	.99
BC(YRP10)	200	176	1:1	.2-.3	.2-.3	.....
F_2	470	163	3:1	.2-.3	.1-.2	.2-.3
Ruby Giant						
F_1	127	0	1:0	.99	.99	.....
BC(RG)	255	0	1:0	.99	.99	.99
BC(YRP10)	176	192	1:1	.1-.2	.3-.5	.1-.2
F_2	184	63	3:1	.95-.98	.8-.9	.90-.95

DISCUSSION.—The virus strains utilized were considered typical of the respective viruses. Variation had been noted previously in the response of particular pepper varieties to different isolates of a single virus, especially TEV and TMV (2). It has not been possible to attach any significance, from a genetic standpoint, to these varietal responses to different isolates of a single virus for lack of distinctive differential varieties.

That the resistance of YRP10 to PVY-N resulted specifically from a gene mutation was not of major concern in these studies. The resistance of this pepper (and its progenies) to this particular virus strain was complete, so far as could be determined, and was shown only by plants homozygous for the single recessive allele. Resolution of the origin of the recessive YRP10 allele for resistance to PVY-N could not be correlated with the occurrence of a similar allele in P11 (and S.C. 46252). The dominant character for pungent fruit inherent in the latter 2 varieties undoubtedly would have been noted in any progeny that derived resistance to PVY-N from these sources. Because the effectiveness of the recessive genetic factors from P11 and YRP10 were indistinguishable with respect to infectivity of PVY-N and occurrence of (susceptible) recombinant genotypes, the respective alleles were considered identical. Therefore, the designation y^a would be equally appropriate for genes that confer resistance to PVY-N, irrespective of their origin from YRP10, P11, or S.C. 46252.

TABLE 3. Results from inoculation with PVY-N of P11 × YRP10 progenies

Progeny	No. of lots	No. of plants Susceptible	No. of plants Resistant
F_1	5	0	88
BC(P11)	12	0	1029
BC(YRP10)	13	0	1403
F_2	57	2[a]	4968

[a] See text discussion.

The susceptibility to TEV-E and resistance to PVY-N of YRP10 progeny plants were sufficient evidence to invalidate the proposed gene designation ey^a (5). Confirmatory evidence was obtained from inoculation of YRP10 × P11 progenies with PVY-N in which no distinctions could be made between the single recessive alleles from the respective resistant varieties. Consequently, only the previous allelic designations et^a+/et^a (6) and Y^a/y^a (4) can be considered appropriate. There remains, however, presumptive evidence of close linkage for the loci of these allelic pairs.

Although only preliminary field trials with YRP10 have been conducted, yield, size, and quality of fruit were comparable to those of the popular commercial variety from which YRP10 was selected. The fruits of YRP10 are not pungent and are of satisfactory size for commercial use. If this selection should not prove commercially acceptable, however, it would be of value as parental material for breeding purposes. The plants of this varietal strain are homozygous for both gene pairs which condition the imperfect localization reaction to TMV (I^pI^p) and determine resistance to PVY-N (y^ay^a). Therefore, lines with the same resistances and sizeable fruit could be selected in the F_2 from outcrosses to many presently available commercial varieties.

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MICROORGANISMS ON CUCUMBER SEEDLINGS

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SUMMARY

A plastic chamber for protecting seedlings from air-borne microflora is described. With this model system, cucumber seeds carrying fewer than 2,000 bacteria/seed gave rise to seedlings with aerial parts having millions of bacteria/plant. High numbers of bacteria also were found in the water reservoir that contained some of the roots. The types of yellow marker bacteria, which were derived originally from soil, were capable of multiplying on aerial parts as well as in the root area.

In the early 1900's, it was recognized that microorganisms could be isolated readily from the surface of the above-ground parts of plants. Noteworthy is the work of Burri (4) and Düggeli (7-10). These men concluded that the bacterial flora on plants is characteristic and differs from that of air or soil. In 1909, Potter (18) described tests in which bacteria and fungi were noted in "leaf impression cultures." In discussing his and the earlier work he stated: "Since this epiphyllous flora is always present upon the surface of green plants, it becomes a matter of considerable interest to determine the part played by these micro-organisms in the ordinary fungoid diseases." Potter felt that the surface organisms were not without some effect and that plant scientists should be aware of their presence. More recent evidence also points to the existence of a specialized microflora on the aerial parts of plants (5, 6, 14, 21, 23). The suggestion has again been made that these organisms might influence plant diseases (5, 12). There are examples in which microorganisms isolated from the upper parts of the plant, when reapplied to the plant, altered the course of disease (2, 3, 11, 13, 17, 22, 26). Ledingham, *et al.* (16) and Simmonds (21) presented strong evidence that bacteria normally carried on wheat seed affected the incidence of seedling blight incited by the fungus *Helminthosporium sativum* Pam., King & Bakke. The use of antagonistic organisms for the control of plant diseases has been reviewed by Wood and Tveit (25).

This paper reports part of an effort to understand the role of nonpathogenic microorganisms on the upper parts of plants. Here one might expect to find epiphytes or "resident" organisms—those organisms that multiply on the plant surface. Also expected to be present would be organisms not possessing this capability; this microflora would likely be on any surface exposed to air. These organisms are termed "casual," as a matter of convenience. Of the 2 groups, the residents would appear more promising for study. One approach would eliminate some of the variables by preventing contamination of the plant by air-borne casuals or by air-borne residents. This has not been done heretofore. The present paper describes a "plastic chamber" for growing seedlings free of the air-borne microflora. Studies are reported on the multiplication of nonpathogenic bacteria on the above-ground parts and in the root area of cucumber seedlings growing in plastic chambers. This instrument has been described in brief (15).

METHODS.—*Plant prints.*—"Leaf impression cultures" (18) or "plant prints" were made by pressing the upper surface of a plant part to the surface of an agar medium in a petri plate. "Replica plates" were used in some tests—a single plant part was employed to print successively a number of plates. Cucumber leaves had essentially the same kinds and numbers of bacteria on the 8th impression in a series of plates as on the 1st, judging by macroscopic inspection.

Quantitative estimation of organisms.—Numbers of bacteria were estimated with an agar-plate dilution technique. For the assay of cucumber seedlings, only the upper portion was used—1-2 cm of the stem, the cotyledons, and the leaf (or leaves). For each assay, 5 of these plant portions were placed in a 500-ml Erlenmeyer flask containing 50 ml of sterile water. Flasks were shaken for 30-45 minutes on a reciprocal shaker. The washings were diluted suitably and used to make 10-fold serial dilution plates, 3 replicate plates for each dilution. The inoculum and *ca.* 15 ml of agar at 42-44°C were added to each plate, the plates swirled, and then incubated at 22-25°C. Colonies were counted at 72-96 hours; if spreading bacterial colonies were present, counts were made at 48 hours or earlier. For seed assays, 20 seeds replaced the plants in the above procedure. Shaking the plants presumably removed only part of the surface organisms. Aside from the tests in which seed was ground, there was no attempt to assay for organisms carried internally.

Fig. 1. Plastic chamber. Left: closed unit. Center: bottom part of chamber consisting of a can, a beaker water reservoir, and a clay pot. Right: upper part of chamber consisting of a plastic covered cylinder with a paper air filter over 1 end and a filter band around the other. Cucumber plants were seeded 10 days before the picture was taken.

Media.—In early work, antibacterial or antifungal media, containing 50 mg/liter of streptomycin or cycloheximide, respectively, were used. When the effort was later centered on the bacteria, it was not found necessary to add cycloheximide. The tests reported herein in detail were performed with nutrient agar ("Difco"—23 g/liter).

Plastic chambers.—Air-borne microorganisms were excluded from the plant by means of a simple plastic-covered chamber (Fig. 1). The apparatus consisted of: 1) a clay pot (*ca.* 8 cm high and 9 cm in diameter) containing damp vermiculite, 2) a bottom of a 1-lb coffee can (*ca.* 7.5 cm high and 12.5 cm in diameter), and 3) a wire cylinder (*ca.* 35 cm high and 11 cm in diameter) wrapped with the transparent plastic film, "Saran" (Dow Chemical Co.). The cylinder was made from 5 x 6 cm wire mesh and was open at the ends. One end was covered by an air filter of 2 layers of paper kitchen towel held with string or a rubber band. The other end was ringed on the outside of the plastic by a cotton band 2 cm thick and 5 cm wide. The cotton may be covered with cheesecloth. The pot was placed in the can and a closed unit formed by slipping the band covered end of the cylinder between the pot and the can. The band formed a seal between the 2 parts and acted as an air filter. The film and the band may be attached to the wire mesh by means of transparent office tape ("Texel," Permacel-LePage's Inc., did not loosen in the autoclave). The plastic film shrank during autoclaving, hence it was necessary to allow for about 20% shrinkage.

Water in addition to that supplied by the moist vermiculite was provided for the plants. A reservoir was made from a beaker; its rim supported the pot rim. The bottom of the pot extended about 1 cm into water in the beaker. The additional water made the opening of the chambers unnecessary for at least 19 days, the longest test period.

In setting up a plastic chamber, the pot of moist vermiculite was placed in a paper sack and autoclaved (120°C) for 2-3 hours. The pot was then put into the water reservoir, and both placed in the can. The chamber was assembled and autoclaved for 1 hour. When the chamber had cooled, it was opened in a transfer room and 20 seeds placed on the surface of the vermiculite. These were covered with sterilized, damp vermiculite. Chambers were taken to the greenhouse and protected from direct sunlight.

Tests were made during the spring and summer months; during warm periods, the house was cooled with an evaporative cooler. The temperature range was 24-32°C. The temperature within the plastic chamber, measured during the time the house was cooled, was 5° higher than the air flowing around the chambers. In the spring, supplemental lighting 18 hours a day was provided by 2-tube, 40-w fluorescent fixtures suspended about 30 cm above the chambers. The relative humidity within the chamber was high, as evidenced by the condensed water on the inside.

Seven days after seeding, cucumber seedlings had 1 leaf, usually about 1 cm in diameter, and the roots usually protruded 1-2 cm from the hole in the bottom of the pot. Nineteen days after seeding, plants had 2 leaves and the shoot apex had reached the top of the chamber. Roots extended about 20 cm from the pot and well into the reservoir liquid, the level of which was then below the bottom of the pot.

The effectiveness of the plastic chambers for excluding air-borne organisms was tested by replacing the pot and reservoir with a beaker of potato agar (per liter: extract from 5 g of sliced, peeled potatoes in 500 ml of water heated 0.5 hour at 120°C; glucose, 5 g; and agar, 17 g) or nutrient agar. Thirty-two plastic chambers were used in 4 tests. No bacteria nor actinomycetes were observed on the media at 14-21 days, the length of the test period. It was concluded, therefore, that these organisms were effectively excluded. In 8 of the plastic chambers there were 1-3 colonies of fungi of the saprophytic types, *e.g.*, of the genus *Penicillium*. This occasional contamination was not considered serious because fungi were occasionally found sporulating within the chamber on the seed coat, which was usually on top of the vermiculite, and because the main effort was subsequently directed to the study of bacteria.

Treatment of seed.—A single commercial lot of "Green Prolific" variety cucumber seed was used. An agar diffusion assay (1) revealed no fungicide.

In some tests seed was treated with sodium hypochlorite ("Chlorox" (The Chlorox Company), 1 in 5

TABLE 1. Number of bacteria indicated on the upper parts of cucumber seedlings from seeds carrying fewer than 2,000 bacteria/seed: plants from plastic chambers

Test No.	Days after planting seed	Bacteria/plant (millions) Chamber 1	Chamber 2
1	14	4	16
2	5	0.1	0.5
	11	20	9
	19	15	4
3	6	2	0.3
	11	4	14
	15	42	2
4	7	105	25
5	7	8	18
	15	25	4
6	7	3	4

(v/v)) at a final concentration of *ca.* 1% active ingredient. Lots of 20 seeds were first dipped in 95% ethanol (to wet seed), soaked in the sodium hypochlorite solution for 16 minutes with an occasional stirring, and then planted. This long treatment time reduced germination markedly; it was used, nevertheless, after it was found that the 5-minute period commonly employed did not sterilize seed.

For treatment with soil, lots of 20 seeds were dipped in a suspension made by adding 1 g of field soil (Wooster silt loam) to 100 ml of sterile water.

RESULTS.—A number of plant print tests with antibacterial or antifungal media and a range of plants were made. Species from the field or greenhouse were used, and tests were conducted in the spring and summer months. A great variety of bacteria and fungi was observed. No consistency appeared in the microbial population. It was recognized that many, or perhaps most, were casual organisms. The decision was made, therefore, to study the resident organisms on 1 plant species, using the plastic chambers to exclude the air-borne flora. Cucumber was chosen. Plants from plastic chambers are here referred to as "protected."

Plant prints indicated that abundant bacteria were on the surface of the aerial parts of protected cucumber seedlings. These organisms could have been derived only from the seed. Colony distribution in the print was uniform, which suggested that the bacteria had multiplied on the surface of the seedling and were not present as a result of a direct contact between the plant part and the seed coat. Distribution on leaves was especially suggestive of multiplication, since leaves were not exposed until the shoot reached a height of 5-10 cm.

To determine if multiplication of bacteria had taken place, dilution assays were made to estimate numbers on the seeds at the time of planting and later on the upper parts of protected plants. Seeds were assayed on 8 occasions. The following are the indicated numbers of bacteria/seed (results from replicate tests conducted at 1 time are separated by semi-colons): 750, 1,750; 250; 42, 62; 80; 97; 50; 105; and 82. In 3 of these tests, additional seeds were ground with a small amount of water in a sterile mortar before assaying. The results were substantially the same as with unground seed. Thus, each seed carried fewer than 2,000 bacteria. In contrast, on the upper parts of protected plants many more bacteria were detected (Table 1). There was usually a large population by 8 days. In some of these tests, the root reservoir liquid, as well as the upper parts of the plant, was assayed. Usually high numbers of bacteria were found. Thus, in test 2 at 19 days there were 14 and 12 million bacteria/plant, respectively, in the reservoirs of the 2 plastic chambers, assuming 20 plants/chamber. In test 3, there were 51 and 8.7 million, and in test 5, there were 11 and 0.1 million at 15 days.

In a given test, leaf prints or dilution assays of protected seedlings revealed relatively few kinds of microorganisms. Usually there were 3-6 colony types of bacteria as determined by macroscopic inspection at the usual counting time. These colonies were white to cream in color, mostly with smooth edges. A few were "spreaders." Chromogenic types were rarely seen. When fungi were present, they were usually *Aspergillus* or *Penicillium* species and commonly only 1 species/chamber. Contamination by fungi from the exterior air or from the seed coat was indicated. Only 2 actinomycete colonies were observed in the course of the work, but more might have been detected with a longer incubation period. Some of the colonies that were macroscopically adjudged bacteria may have been yeasts; high levels of these organisms have been found on older plants (6).

From these tests it was concluded that the abundant resident bacteria on the above-ground parts of the seedlings were derived from the seed. Bacteria in the root reservoir were derived from the same source. Presumably the bacteria multiplied as the plant grew, although in the root area another path for growth may have been in the water interfaces of the vermiculite. In any event, the nutrients for supporting bacterial growth in the root area must have come from root exudates, for no other nutrient was added to the vermiculite.

Results confirming these observations were obtained in experiments in which yellow marker bacteria were added to the seed and later detected on the upper parts of protected cucumber seedlings and in the root reservoir. The marker organisms, numbered 481 and 489, were originally isolated from the upper parts of protected cucumber seedlings that grew from seed dipped in a soil suspension. Each marker organism produced a characteristic colony type and color. To treat seed with a marker, seed was dipped momentarily in a dilute cell suspension from a pure culture of the marker bacterium, blotted with sterile filter paper, and planted in the usual way in plastic chambers. The number of marker cells on the treated seed was estimated by shaking the blotted seed in

TABLE 2. Numbers of yellow marker bacteria on upper parts and in the root reservoir when plants were grown from seed treated with marker bacteria: plants grown in plastic chambers

Test No.	Marker bacterium designation	No. of marker bacteria added/seed	Days after seeding	Marker bacteria on upper parts of plants (millions/plant)		Marker bacteria in root reservoir (millions/plant)	
				Chamber 1	Chamber 2	Chamber 1[a]	Chamber 2[a]
1	481	Not determined	14	12	16	[b]	[b]
3	481	1.2×10^6	15	36	16	5.6	33
4	481	<100	7	4	20	2.5	13
5	481	<1,000	7	82	28	69	18.5
6	481	<1,000	7	29	28	0.08	5.6
3	489	2.5×10^6	15	3		12	
4	489	<100	7	16	15	8.3	<0.8
5	489	<2,000	7	12	36	51	1.5
6	489	<100	7	3		2.5	

[a] Assuming 20 plants/plastic chamber.
[b] Too numerous to count in dilutions that were used.

water as for the regular seed assay. Results of the tests with markers are found in Table 2. Data for some of the tests in Table 2 may be compared with those in Table 1, which contains data from the parallel series of protected seedlings that grew from seed not soaked in a suspension of a marker organism.

Attempts were made in plastic chambers to produce plants that carried no resident bacteria. Sodium hypochlorite was used for treating seed. As evidenced by plant prints or by dilution assays, sterile plants were obtained in 2 of 10 plastic chambers examined. Abundant bacteria were found on the plants in the others. There were usually 1-2 colony types/chamber instead of 3-6 types observed in parallel tests with plants from untreated seeds. In 3 tests with 6 plastic chambers, untreated seeds were dipped in a soil suspension, and more than 10 colony types/chamber were discerned. Chromogens, especially various shades of yellow and orange, were notable. Yellow marker bacteria 481 and 489 were selected from among these organisms.

DISCUSSION.—The present work presents additional evidence for the existence of a resident microflora on the surface of aerial parts of plants. Effort was centered on bacteria, but other types of microorganisms also may be present (5, 6, 14). How many kinds of resident bacteria there are, and how specific they may be for a given plant species, remain to be determined. It is concluded that these organisms may be carried on the seed, as first noted by Düggeli (8), or that they may be derived from the soil, as indicated in the experiments with the yellow marker bacteria. It seems likely also that some of the resident flora may be air-borne on debris or in rain water.

In the present work, high numbers of bacteria derived from the untreated seed were observed in the root reservoir. When the yellow marker bacteria, which multiplied on the upper parts of the plant and which came originally from the soil, were placed on the seed they too were noted in great numbers in the root reservoir. Under conditions of the tests, it is likely that the organisms found in the reservoir would also be in the rhizosphere. If this is true, the present work adds to the evidence that rhizosphere organisms may originate on the seed (8, 20), or on both the seed and soil (19, 24). It also suggests that some of the rhizosphere bacteria may be derived from residents of the aerial parts of plants.

The experiments were, of course, conducted with a model system. It would be expected that, in the plastic chambers, the high relative humidity and a film of water on the surface of the upper parts of plants would favor multiplication of bacteria. There also may be a high relative humidity and free moisture on plants for extended periods of time in nature, especially during certain seasons in parts of the world. Resident organisms would be expected to have periods of activity or inactivity. Conditions for root growth in the plastic chamber also were different from those in nature. For example, the only microorganisms present were those from the seed. Further, and in contrast to soil, the vermiculite has little nutritional value, either for the plants or for microorganisms.

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TREE NUTRITION AND FIRE BLIGHT DEVELOPMENT

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SUMMARY

Natural occurrence of fire blight in 1959 in a pear orchard receiving N-P-K factorial fertilizer treatments since 1953 indicated that extra nitrogen applications increased blight susceptibility. The addition of potassium with nitrogen caused no further increase in blight severity. The addition of phosphorus and of phosphorus and potassium, with the nitrogen, did cause substantial increases in susceptibility.

In a 2nd orchard where a previous report showed good soil drainage correlated with lesser blight development, terminal buds matured earlier in the better-drained area. The trend was for more succulent shoots when phosphorus or phosphorus and potassium were added with the nitrogen. These differences were statistically significant only when phosphorus, phosphorus and potassium, or even potassium alone was added to the mulch and nitrogen treatment.

Inoculations of apple trees growing in sand culture with nutrient differentials supported the field results. Additions of potassium with nitrogen caused little or no increase in blight susceptibility, but additions of phosphorus or phosphorus and potassium resulted in a marked increase in susceptibility.

It is well known that high vigor of pomaceous fruit trees favors initiation of infection by *Erwinia amylovora* (Burr.) Winslow, *et al.* and subsequent development of fire blight. Many pear orchards in New York State are maintained in low vigor to prevent severe disease development. Fruit production is often so low that crops are unprofitable. Some orchards, however, are kept in high enough vigor to produce well without

severe blight development. A study is in progress to determine why such wide differences in severity of this disease exist in the same area. In this, the 2nd report on this study, additional data are given on the relation of tree nutrition to fire blight development. Also, this paper reports growth data pertinent to some of the fire blight data in the 1st paper (3).

REVIEW OF LITERATURE.—Based on many years' observations on development of fire blight of apple and pear in New Jersey, Blake (1, 2) recommended that susceptible varieties be planted on deep, fertile, well-drained but not droughty soil. His explanation was that anything that restricts the root system or its function, such as very dry or very wet conditions and concentration of fertilizers in a restricted area under the tree, will contribute to high susceptibility. These conditions and others, including heavy crops, excessive pruning, and hot, dry weather, cause a low carbohydrate content of the tissue and a consequent high level of susceptibility to fire blight. Such a condition, he suggested, may exist where growth is excessively rapid and succulent or is slow and weak.

A survey of naturally occurring fire blight in New York orchards (3) showed that soil drainage has a significant effect on fire blight severity—symptoms were generally less severe in trees on well-drained soils than on poorly drained soils. Inoculations made on shoots from orchard trees supported this conclusion. The same experiment indicated that mulch treatments favored blight development. Additions of phosphorus had no apparent influence on susceptibility to blight, but potassium additions resulted apparently (although not statistically significant) in reduced susceptibility. In this orchard, growth and fruit production responses were obtained with potassium additions. Leaf potassium content was less in trees in the wet areas than in those in better-drained areas; blight development was greater in shoots from trees in the wet area.

EXPERIMENTS. — *Orchard experiments.*—1) *Horn Farm (sod).*—This experiment, described earlier under the same designation by Fisher, *et al.* (3), was started in 1953 in a 12-year-old Bartlett pear orchard. It was designed as an N-P-K 2×2×2 factorial experiment with eight 2-tree replications. A single application of 20 lb of superphosphate was made to each plus-phosphorus tree in the fall of 1953. The plus-potassium trees received 5 lb of potassium chloride in the spring of 1954 and 7 lb of potassium sulfate in the spring of 1957. Over-all applications of urea sprays were made by the grower in 1954 and 1955, and he applied 1.5-2.0 lb of ammonium nitrate in 1956, 1957, and 1958. In 1959, the only nitrogen applied was to the plus-nitrogen trees, which received 2 lb of ammonium nitrate each spring.

Fire blight development was determined in 1959 at the end of the growing season by measuring the length of each lesion and the diameter of the branch on which it occurred. The product of lesion length × the mean diameter of the branch or twig was used to indicate severity. Where the lesion progressed into a larger supporting branch or scaffold limb, the product of lesion length × diameter was determined for each portion and these values added. From these figures, an average severity was determined for individual trees. Only lesions resulting from blossom infections were included, limiting the comparison to infections that occurred at approximately the same time on all trees.

2) *Sodus Fruit Farm.*—This experiment, also described earlier under the same name, was designed as a mulch-N-P-K 2×2×2×2 factorial with eight 2-tree replications. The Bartlett pear trees were approximately 12 years old at the beginning of the experiment. Phosphorus, potassium, and experimental nitrogen applications were made as in the Horn Farm experiment. The orchard received an over-all application of 1.5-2.0 lb of ammonium nitrate/tree each spring. In 1957, 0.75 lb of potassium chloride was added to this over-all application. Each mulched tree received 1 bale of straw in 1954, 1955, 1957, and 1959.

To supplement the fire blight records taken in this orchard in 1957-58 (3), a study was made in 1959 of the growth stage of the terminals. Records were made on 3 replications on June 30 and on 2 additional replications 3 days later. These 5 replications were distributed throughout the orchard, so a complete sampling was obtained of trees in all soil conditions in the planting. Five terminals on each tree were rated with respect to growth stage: 1 = succulent; 2 = terminal bud forming. An average figure was determined for each tree, then the values for the 2 trees in a given treatment replication were added together for the statistical calculation.

Greenhouse sand culture experiment.—In 1955 a greenhouse experiment was begun using 1-year-old East Malling VII apple trees. These were planted in a mixture of coarse and fine white sand in 1-gal glazed crocks with an opening in the bottom for drainage. Modifications of Hoagland's solution (4) were used to supply mineral nutrients, the full concentration in the following listing designated as 1H. The nutrient treatments were as follows: O = Hoagland's complete solution with N, P, and K all at 0.1H; N = N at 1H and P and K each at 0.1H; NP = N and P at 1H and K at 0.1H; NK = N and K at 1H and P at 0.1H; NPK = all nutrients at 1H.

Five hundred ml of solution were applied to each tree twice weekly. Between nutrient feedings, the sand was flushed with distilled water. Two months after the trees had reached the green tip stage, the terminal shoots were inoculated by hypodermic injection of a suspension of *Erwinia amylovora.* Five trees, randomized in a Latin Square arrangement on the greenhouse bench, were used in each treatment. Measurements of growth and of blight lesions were made a month after inoculation.

These trees were allowed to grow on the various nutrient treatments for another 2 months after the

TABLE 1. Severity of naturally occuring blossom blight on Bartlett pear trees given supplementary applications of nitrogen, phosphorus, and potassium (Horn Farm, 1959)

Treatment[a]	Severity of infection	
	Mean rating[b]	Relative to NPK treatment
0	13.9	15
N	48.9	53
P	13.1	14
K	14.2	15
NP	72.4	78
NK	51.0	55
PK	21.8	23
NPK	93.1	100

[a] Applications made in addition to regular applications of nitrogen made by grower.

[b] Mean of products of length of lesion and diameter of branch for five 2-tree replications. LSD: 0.05–47.7; 0.01–64.3.

blight became arrested. Blighted stems were then removed and the trees placed in cold storage. In 1956, the same trees were used for a repetition of the experiment, so that all trees were given a 2nd year of differential mineral nutrition. Inoculations, made 2½ months after green tip, were made on the largest shoot on each of the 25 trees. Leaf samples from 1 or 2 shoots on each tree were taken the 2nd year, dried, and analyzed for total organic and ammoniacal nitrogen by the Kjeldahl method.

RESULTS.—*Blight infection in the Horn Farm experiment.*—In 1959, blossom infections were distributed in 5 complete replications: 1 or more infections occurred on at least 1 of the 2 trees in each replication of each treatment. Only 2 of the 2-tree replications had a single infection on only 1 tree—usually at least 5 or 6 lesions were measured on each tree. The mean severity of blight infection for each tree was determined. Where infections occurred on both trees of the replication, an average of the 2 values was used for the statistical analysis. Where only 1 of the 2 trees in the replication bore infections, the value for that tree was used.

Infection on trees receiving the complete nutrient treatment was the most severe; most of this increase was due to N and P, for infection of trees receiving the NP treatment was only slightly less severe than that of trees receiving the complete treatment. Of the single-element applications, only that containing N increased susceptibility; the response was about the same from NK as from N alone; and PK increased susceptibility only slightly. Even the untreated checks received nitrogen applied by the grower, who applied N to all trees in a small amount sufficient to produce a moderate crop.

Growth stage of terminals in Sodus Fruit Farm experiment.—Results are given not only for the different treatments, but also for the different replications (Table 2), because the soil generally ranges from well drained in replication I to poorly drained in replication V.

Trees in the N + mulch treatment were significantly less succulent (greater number of terminal buds formed) than those that received the NP or NPK without mulch or mulch + NK, NP, or NKP. Those receiving N and NK without mulch were not statistically more succulent than those receiving N + mulch, although their growth stage rating was less.

The only significant difference between replications is between replications II and V, but the trend is for more advanced maturity at replications I and II with greater succulence at the other 3 replications. This is in spite of the fact that in some years fruit size has been greater in the well-drained area of the orchard (3) and the trees generally larger and more vigorous.

Greenhouse sand culture experiment.—From measurements of shoot growth and the extent of blight, the percentage of shoot growth blighted was determined. Trees given the 0 treatments had very little blight. This level of nutrition also resulted in less growth.

The addition of potassium to nitrogen had little influence on blight development, but the addition of phosphorus (significant in 1956) or phosphorus and potassium (significant in 1955-56) resulted in increased blight susceptibility.

Leaf analyses for total nitrogen (Table 3) show that all the + N treatments significantly increased (0.01 level) leaf nitrogen over the 0.1 Hoagland's solution (0 treatment). Leaf nitrogen in the N, NP, and NPK treatments were equal, but leaf nitrogen content of NK samples was significantly less (0.05 level) than NP as shown by the Wilcoxon method of ranking (5).

TABLE 2. Growth stage, on June 30 and July 3, of terminals of Bartlett pear (Sodus Fruit Farm) given supplementary applications of nitrogen, phosphorus, and potassium, with and without straw mulch (lower value = more succulence)

Treatment or replication	Mean growth stage of terminals[b]	
	Without mulch	With mulch
Treatment[a]		
N	3.20	3.52
NK	3.16	3.04
NP	3.04	3.00
NPK	3.08	2.96
Replication[c]		
I	3.250	
II	3.325	
III	3.000	
IV	3.075	
V	2.975	

[a] Treatments were in addition to grower application of small amounts of nitrogen.

[b] Each figure is the mean for 5 replications; value for each replication was the sum of 2 single-tree values. Each value was the average for 5 terminals, rated 1 if succulent and 2 if terminal buds were forming. The lower the mean, the more succulent were the shoots. LSD for treatments, 0.42(0.05) and 0.57(0.01); for replications, 0.334(0.05) and 0.450(0.01).

[c] Drainage ranges from good at replication I to poor at replication V.

TABLE 3. Growth, blight development, and nitrogen content of leaves on apple trees receiving differential nutrient treatments in sand culture and inoculated with *Erwinia amylovora*

Treatment[a]	1955 shoot growth		1956 shoot growth		Leaf nitrogen content in 1956 (% dry weight)
	Length[b] (cm)	Percentage infected[c]	Length[d] (cm)	Percentage infected[c]	
O	21.6	9.7	63.2	0.6	1.77
N	28.0	40.1	79.8	30.5	2.76
NP	36.6	48.6	79.4	43.7	2.77
NK	32.8	34.2	74.0	32.0	2.58
NPK	45.2	79.3	91.2	42.5	2.75
	LSD: 0.05	21.5		10.8	
	0.01	29.6		14.9	

[a] NPK = full strength Hoagland's solution. Deletion of a symbol means that the element was present at 0.1 of the concentration in the full strength solution.
[b] Mean for 5 shoots—the single new shoot on each of 5 trees.
[c] Percentage of linear length of the inoculated shoot blighted, mean of 5.
[d] Mean for 5 shoots—the largest new shoot on each of 5 trees.

DISCUSSION.—At the Sodus Fruit Farm orchard, where there was a response in yield and fruit size to potassium additions and where potassium content of the leaves on untreated trees was low, there was an indication of a lesser susceptibility to blight on trees receiving extra potassium (3). In the same experiment, there was clearly greater susceptibility to blight in the poorly drained part than in the well-drained part of the orchard. This was correlated with prolonged growth into the summer and a lower potassium content in the leaves. In the experiment on the Horn Farm, where potassium was not limiting, the addition of potash alone or with extra nitrogen did not influence disease susceptibility. It did tend to increase natural severity when combined with high levels of both nitrogen and phosphorus. One may tentatively conclude, therefore, that potassium may be added if needed for growth without danger of increasing susceptibility to fire blight, unless nitrogen and phosphorus are both at high levels.

Phosphorus alone did not increase disease development in the Horn Farm experiment but did when added to extra nitrogen. This may be explained partly by an increase in leaf nitrogen in this orchard when phosphorus was added along with extra nitrogen. These results tend to discourage the addition of phosphorus. In the Sodus Fruit Farm experiment where the natural level of phosphorus was lower, the addition of phosphorus did not increase susceptibility to disease as measured by artificial inoculation of detached shoots (3).

The results of the greenhouse nutrition experiment tend to support the field experience. Both indicate that of the 3 major nutrient elements, nitrogen is by far the most important in affecting susceptibility to fire blight. Phosphorus appears to be 2nd in importance; however, the actual effect of any element in a given orchard depends not only upon the amount of that element present but also on the amounts of the other 2 elements and on factors such as drainage. The whole question of nutrient balance in relation to susceptibility to fire blight deserves intensive study.

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ANTIHOST SERUM IMPROVES PLANT VIRUS PURIFICATION

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SUMMARY

Serological detection of host contaminants in a virus preparation which supposedly had been purified by conventional methods led to a study of serological means of eliminating such host constituents. It was shown that normal rabbit serum, as well as antiserum against the juice of healthy cowpeas, inhibits the infectivity of some viruses. By means of sodium sulfate fractionation, alcohol precipitation, and electrophoretic separation, antibodies were prepared against healthy host antigens, free from substances which adversely affect virus infectivity. Antiserum against healthy cowpea juice, treated to remove substances which inhibit virus infectivity, was used to react with and remove host contaminants in a semipurified preparation of a supposed tobacco necrosis strain. The virus was separated from the excess unreacted antibodies by density gradient centrifugation. This approach to virus purification is useful when host substances inhibit virus infectivity or when host substances have chemical and physical properties similar to those of the virus, making purification difficult.

The separation of a virus from cell components of the host is necessary for the study of some of its properties. The ultracentrifuge and electrophoresis apparatus generally have been useful here. With some hosts, however, where large amounts of substances have sedimentation properties overlapping those of viruses, the ultracentrifuge has limited effectiveness. The Tiselius electrophoresis apparatus is limited in the number of components it can separate; it requires far higher concentrations of virus for convenient separation than are practically obtainable with many plant viruses. Other methods of separating virus from normal host cell components which employ selective destruction, such as the use of high temperatures or denaturing solvents, frequently cause serious losses of infectivity, and may be less effective in removing contaminating host substances than may be inferred from commonly used tests for purity. Antihost sera (antisera prepared by injecting animals with crude juice of a noninfected host plant) are rarely used as a means of testing for virus purity, yet they can readily demonstrate contamination of apparently purified virus preparations by certain host constituents.

Antihost sera were used as early as 1937 by Dunin (6) to prepare antisera against a plant virus which would not react with host juice. To accomplish this, he added antihost serum to infective juice. Normal host antigens were precipitated from the mixture by the antihost serum. The supernatant from this reaction was injected into animals, producing antiserum which would react with the virus in crude infective juice, but not with normal juice components.

It may seem strange that this principle of eliminating host constituents by serological means has not been used for virus purification, because it is a highly specific means of removing host antigens. Undoubtedly, the reluctance to add large amounts of a foreign protein to a system from which one is attempting to eliminate contaminants has been 1 reason. Reports that blood, milk, and many other natural products inactivate viruses (5, 8, 11, 16) may also have discouraged efforts at virus purification by serological means. The following study—to determine whether the theoretical specificity of virus purification by serological means could be practically utilized—was motivated by the discovery that a virus which had been apparently purified by conventional means was still grossly contaminated by host antigens.

Apparently purified virus contains abundant host constituents.—This work was the result of efforts to purify a supposed tobacco necrosis strain which had been isolated from Salinas, Calif., soils. Bawden and Pirie (1, 2) have shown that the name "tobacco necrosis virus" embraces several unrelated viruses. The principal virus in this study resembles published descriptions of so-called tobacco necrosis virus in most respects, but differs in some prominent details. A description of its properties, occurrence, and ecology is the subject of a separate unpublished paper. In the absence of opportunities to compare this virus directly or serologically with other claimed tobacco necrosis isolates, the virus strain in this study is designated "TNV," without implying the nature of relationship of this isolate to others. Because this virus did not multiply well in tobacco, the primary leaves of 15-day-old cowpea plants were inoculated. Three days after inoculation, lesions appeared on the inoculated leaves.

In an attempt to purify this virus, the infected leaves were harvested on the 4th day after inoculation and frozen. The frozen leaves were ground in the presence of 3% K_2HPO_4 (fresh weight of the leaves) and the juice of the ground mass was filtered through cheesecloth. The crude juice was centrifuged for 10 minutes at 9,000 *g*. The clarified juice was centrifuged for 90 minutes at 80,000 *g*. The resulting pellet was resuspended in 0.05 *M* phosphate buffer, pH 7.5, at 0.1 the volume of the original juice. This preparation underwent another cycle of low-speed and ultracentrifugation. The resuspended pellet from the last ultracentrifugation was subjected to density gradient ultracentrifugation, using a slight modification of Brakke's (3) method.

For the density gradient centrifugation, a series of sucrose (cane sugar, table grade) solutions was prepared in 0.05 *M* phosphate buffer, pH 7.5, with the following concentrations of sucrose by weight: 60, 50, 40, 30, and 20%. One-half-ml portions of each solution were layered successively in tubes for a Spinco 39 swinging bucket rotor. One-ml portions of the virus preparation, treated as described in the previous paragraph, were layered on the sucrose columns. The tubes were centrifuged for 90 minutes at 80,000 *g*.

The turbid bands which appeared in the sucrose columns after the centrifugation were removed separately, and tested for virus infectivity by inoculating the primary leaves of 15-day-old Beka bean seedlings with part of each fraction. The layers of several runs showing the highest infectivity were pooled and dialyzed against 0.05 *M* phosphate buffer, pH 7.5, to remove the sucrose.

The virus purified in this way was used to prepare antiserum. Two rabbits were injected intramuscularly 3 times at weekly intervals with 1 ml of the virus emulsified with Freund's (Difco-Bacto) adjuvant. Two weeks after the last injection, blood was drawn. The pooled serum from this blood was used in drop-scale precipitin serological tests according to van Slogteren's method (14). It is normal in such tests to use healthy juice as a control. The serum showed such a strong reaction to antigens in the normal cowpea juice, that it was necessary to allow the serum to react with at least 5 vol of host juice and centrifuge off the resulting precipitate before the reaction between the virus and the virus-specific antibodies could be observed. Apparently, the pure virus, which had been used to prepare the antiserum, actually was contaminated with considerable quantities of host antigens. This inference was substantiated by the results of agar diffusion serology, according to van Slogteren's (15) modification. While a relatively faint virus reaction line appeared, there were 2 dense host reaction lines.

To determine the antivirus titer of the antiserum, it was absorbed, as described in the previous paragraph, at a ratio of 1 vol/serum to 9 vol/crude healthy cowpea juice. Normal serum, similarly absorbed, served as a control. The absorbed sera were tested against TNV and healthy cowpea juice which had been subjected to 1 cycle of low-speed and ultracentrifugation. The pellets from the ultracentrifugation were resuspended in 0.05 *M* phosphate buffer, pH 7.5, to make 0.1 vol of original juice. As Table 1 indicates, a serum dilution titer against the virus of 1:320 was obtained.

Because ultracentrifugation alone had failed to eliminate serologically detectible host substance contaminants of the virus, an effort was made to eliminate them by selective inactivation. A portion of TNV which had undergone the repeated ultracentrifugation previously described was heated for 10 minutes at 60°C. This portion then was tested for infectivity and for the presence of host contaminants. More than 99% of the infectivity had been destroyed without eliminating host antigens. Thus, certain of these contaminating host antigens were more thermostable than the virus.

Effect of anticowpea and normal rabbit serum on virus infectivity.—These failures to eliminate serologically detectable host constituents from virus by conventional means stimulated efforts to remove the contaminating host substances by serological means. Anticowpea antiserum was prepared by injecting a rabbit with crude healthy cowpea juice with the same schedule and adjuvant as previously described in the preparation of anti-TNV antiserum. A portion of crude juice from cowpea infected with TNV was added to 4 vol of the anticowpea serum. A similar portion of infective juice was added to normal rabbit serum. A 3rd portion of infective juice, to which 0.9% NaCl had been added, served as a control. The 3 preparations were stored in a refrigerator overnight.

After incubation, the 3 preparations were assayed to compare the relative effect of the 3 treatments on the infectivity of the virus. The assay inoculations were carried out on the primary leaves of 15-day-old Beka bean seedlings, with 6 plants/treatment. Immediately before inoculation, all the juice portions were diluted with 0.05 *M* K_2HPO_4 to a final dilution of 1:100 of the original juice concentration. On the basis of previous experience, this dilution was necessary to reduce the numbers of lesions on the control leaves to countable levels. One primary leaf of each plant was inoculated with virus subjected to 1 of the 2 treatments, and the other leaf was inoculated with the control infective juice. The total number of lesions on leaves inoculated with diseased juice plus anticowpea serum was 10, compared with 577 on the control leaves inoculated with diseased juice plus 0.9% NaCl. There were 21 lesions on the leaves inoculated with diseased juice plus normal serum, compared with 872 lesions on the control leaves.

A similar experiment was repeated with tobacco rattle and tobacco mosaic viruses, in which only the infectivity of virus plus normal serum was compared

TABLE 1. Microprecipitin reaction of TNV against absorbed antiserum[a]

		TNV			Healthy cowpea juice		
		1:1	1:2	1:4	1:1	1:2	1:4
Anti-TNV serum absorbed 1:10	1/80	+	+	+	—	—	—
	1/160	+	+	+	—	—	—
	1/320	+	+	?	—	—	—
Normal serum absorbed 1:10	1/80	—	—	—	—	—	—
	1/160	—	—	—	—	—	—
	1/320	—	—	—	—	—	—

[a] The virus preparation had undergone 1 ultracentrifugation. The pellet had been resuspended in 0.1 vol of original juice. All dilutions of host juice, infected juice, normal serum, and antiserum were made with 0.9% NaCl. Both antiviral serum and normal serum were absorbed 1:10 with juice of healthy cowpeas before use, to remove antibodies against the host.

with the infectivity of virus plus 0.9% NaCl. The general arrangement of experiments was the same, except that it was necessary to dilute tobacco juice with tobacco mosaic virus 1:10,000 to get a reasonable lesion count. For the assay of tobacco mosaic virus, primary leaves of pinto bean seedlings were inoculated. Beka bean seedlings were inoculated with tobacco rattle virus. Tobacco rattle virus was fully as sensitive to inhibition as was the TNV strain. Only 7 lesions appeared on the leaves inoculated with tobacco rattle virus to which normal serum had been added, compared with 2,326 lesions on the control leaves inoculated with tobacco rattle virus plus 0.9% NaCl. Tobacco mosaic virus, however, seemed relatively unaffected by blood serum. There was no consistent difference in the number of lesions in the presence or absence of normal serum.

Preparation of semipurified antihost antibodies.— The fact that normal serum, as well as antiserum against healthy host, affected virus infectivity indicated that the inactivating substances of blood serum were not necessarily identical with serum antibodies, and that they might be separated from each other. Of the general principles which have been applied by immunologists to the separation of antibodies from other blood components, the only specific preparative separation involves reaction of an antigen with antiserum antibodies, and dissociation of this antibody-antigen complex with strong salt solution after separating the complex from accompanying serum components by centrifuging and washing. This procedure has not been useful where proteins were the antigens (10). It has been demonstrated by dilution that the reaction between tobacco mosaic virus and TMV antibodies is reversible (12), but the equilibrium is so much in favor of the TMV-antibody complex that the antibody released on dilution was less than 1% of the total. Other methods, involving differential solubility in various solvents, differential mobility in an electrical field, or other chemical or physical properties, are not so highly specific, but are more widely usable. The fractionation procedure used involved Na_2SO_4 precipitation of the globulin fraction according to a modification of the method of Heidelberger, *et al.* (9), and fractionation of the globulins with alcohol-buffer mixtures, followed by electrophoresis. In the course of the fractionation, each fraction was tested for the presence of antibodies, and as described later, those fractions showing antibody activity were tested for relative inhibition of virus infectivity.

To accomplish fractionation with Na_2SO_4, crude anticowpea antiserum was centrifuged at 9,000 *g* for 15 minutes to eliminate gross debris. This serum was warmed to 37°C, and an equal volume of Na_2SO_4 solution saturated at 37°C was added to it. The resulting precipitate was centrifuged down at 99,000 *g* for 15 minutes in a centrifuge rotor which was allowed to warm to approximately the same temperature. (If centrifuging is done in a cooled rotor, Na_2SO_4 crystallizes out, while the globulin fraction partly redissolves. The resulting pellet is a mixture of Na_2SO_4 crystals and part of the globulin fraction.) The supernatant fluid, which contained most of the pigment and albumin, was decanted and discarded. The pellet was redissolved in water to a volume approximately 0.2 of the original serum. The redissolved material from the pellet was precipitated again with an equal volume of saturated Na_2SO_4 solution, centrifuged down again, the supernatant liquid decanted off, and the pellet redissolved, as before. The solution was dialyzed against 0.9% NaCl to remove contaminating Na_2SO_4.

The serum fraction at this stage was colorless and entirely soluble. Electrophoretic analysis showed that the albumin component had been entirely removed, and semiquantitative seriological tests with crude cowpea juice indicated that no appreciable antibody activity had been lost.

The anticowpea antiserum was further fractionated by a simple modification of the alcohol fractionation methods used by Cohn and his co-workers (4) for the separation of human blood components. Ethyl alcohol was added to portions of 0.02 *M* phosphate buffer, pH 7.0, to make solutions 10, 15, and 20% in alcohol. The serum fraction from the Na_2SO_4 precipitation was dialyzed with stirring successively in each alcohol solution for 12 to 16 hours. The dialysis in 10% was carried out at 0°C; and, in the higher alcohol solutions, at −5°C. The precipitate which formed on dialysis in each solution was centrifuged before dialyzing the supernatant solution in the next more concentrated alcohol solution. Each precipitate was then redissolved in cold 0.9% NaCl, and dialyzed against more saline to remove the alcohol residue. Before further tests, each fraction was brought up to the volume of the original serum by the addition of saline.

Considerable denaturation of some serum components occurred during dialysis against 10% alcohol, because a large part of the precipitate did not resuspend in saline. The precipitates from dialysis in 15 and 20% alcohol solutions redissolved easily and completely. Each of these fractions was tested for antibody activity against the crude juice of healthy cowpeas. Both the 10 and 15% alcohol fractions contained antihost antibodies, while the 20% fraction had none.

The final fractionation step involved electrophoretic separation in a Tiselius separation cell. (The kindness of Dr. William N. Takahashi in performing electrophoretic runs is gratefully acknowledged.) By the use of this cell, the serum could be separated into 3 fractions: a fast moving component which was electrophoretically homogeneous, a slow moving component which was also electrophoretically homogeneous, and a middle fraction which consisted of a mixture of components. For the electrophoretic separation, the 15% alcohol serum fraction was used. The fast, slow, and intermediate parts of this serum were tested separately for antibody activity against healthy cowpea juice. In accordance with the results of Kabat (10),

TABLE 2. Effect of different treatments of anti-healthy cowpea serum (rabbit) on infectivity of TNV

Treatment of anti-healthy cowpea serum	No. of lesions		Ratio treated: Control
	Control	Treated	
Na_2SO_4	2288	310	0.135
10% alc.	3718	1689	0.45
15% alc.	2937	1160	0.40
Electrophoresis[a]	2754	2840	1.03

[a] Slow component. Although electrophoretic separation removes all inactivating substances, the volumes obtainable are small, and serum prepared by alcohol precipitation may serve many practical needs.

the slowest, or γ-globulin, component proved serologically active. The middle component also was serologically active, but was not used for further work because of its mixed composition.

Purified antihost serum fractions do not inhibit virus infectivity.—In the course of the fractionation procedure described above, antihost antibody activity had been observed in the anticowpea serum fraction precipitated by half-saturated Na_2SO_4, by serum fractions which had been further precipitated in 10% ethyl alcohol and 15% alcohol, and by the 15% alcohol fraction which had undergone electrophoretic separation. These serum fractions were now tested for relative freedom from substances which affect virus infectivity. For this purpose, each fraction was used at the volume of the original serum from which it was derived. Juice from primary leaves of cowpeas infected with TNV was mixed with portions of the following fractions of the anticowpea serum: Na_2SO_4 precipitate, 10% alcohol precipitate, 15% alcohol precipitate, and the slow component from the electrophoretic separation. Two vol of each serum portion were mixed with 1 vol of infective juice. One vol of infective juice, mixed with 2 vol of 0.9% NaCl, served as a control. Six Beka bean seedlings were used to assay each preparation. One primary leaf of each bean seedling was inoculated with the control juice. The other leaf was inoculated with juice treated with 1 of the serum fractions. Juice of all fractions was diluted with 0.05*M* K_2HPO_4 to 100 × vol of original crude juice immediately before inoculation. Table 2 summarizes the numbers of lesions found with the various treatments.

These results show that it was possible to separate the anticowpea antibodies from substances in the serum which interfere with virus infection. The Na_2SO_4 fractionation was relatively ineffective in removing these substances, but was valuable as a preliminary step to eliminate haemoglobin, albumin, and possibly other extraneous substances. The alcohol precipitation apparently accomplished considerable removal of inhibiting substances, but there seems to have been little difference between the 10 and 15% alcohol fractions. Only the electrophoretic separation accomplished complete removal of inhibiting substances. Because crude serum was not subjected to electrophoresis, it is not known whether electrophoresis alone would have accomplished such separation.

Use of purified antihost antibodies may improve purification of certain viruses.—The general purpose of the foregoing procedure had been the elimination of host substances from TNV without excessive infectivity loss. This has been accomplished. The remaining task was to determine if the virus could be readily separated from the unreacted antibodies. Because rabbit antibodies have much smaller sedimentation constants (10) than those of the known plant viruses (13), it seemed feasible to separate the unreacted excess antibodies from the virus by density gradient centrifugation. Accordingly, the electrophoretically separated antibody fraction of anticowpea antiserum was used with TNV. The slow fraction from electrophoretically separated anticowpea antiserum, which had been prepared as described in the previous section, was centrifuged for 1 hour at 80,000 *g* to remove aggregates formed during the antibody purification procedure which would have sedimentation values approaching those of the virus. The supernatant liquid, which tests proved serologically active against healthy cowpea juice, was used in the virus purification.

Two vol of this antibody preparation were incubated overnight with 1 vol of TNV which had been partly purified by being subjected to 2 cycles of slow speed (9,000 *g*) centrifugation and ultracentrifugation (80,000 *g*). A heavy precipitate formed on storage in the refrigerator overnight. The turbid suspension was shaken up and layered on sucrose columns which had been prepared, as previously described, for density gradient centrifugation. After centrifugation for 1 hour at 80,000 *g*, 3 bands appeared in the column. The topmost diffuse band, which was interpreted as unreacted antibody, and the bottom layer were not infective. The middle layer, which was about at the 30%-sucrose level, was infective. After dialysis, electron microscopy showed this layer to consist of uniform spheroidal particles, approximately 30 mμ in diameter.

DISCUSSION.—Separation of the substances in rabbit blood which affect the infectivity of plant viruses from the antibody component is a problem in protein fractionation. Probably, for many applications in plant virus purification, antihost serum prepared by Na_2SO_4 precipitation, followed by dialysis against 15% alcohol solution, would be sufficiently free from these inactivating substances. The infectivity loss of only 50% experienced in the case of TNV is still very good compared with the greater infectivity losses experienced with some other virus purification procedures. For other purposes, or other viruses, electrophoretic separation may be required. The chief disadvantage of the electrophoretic purification of antihost antibodies, apart from availability of the equipment, is the relatively great effort required for a small amount of antihost antibody. Application of the sophisticated methods described by Edsall (7) for human plasma protein separation may be required for the production of rabbit antihost antibodies in the quantity and degree of purity sufficient for certain types of virus purification.

If purified antihost antibodies can be produced in sufficient volume, they should be useful where virus purification has been unsuccessful or uncertain. Apparent virus instability may in some cases simply reflect the action of destructive enzymes from the disrupted cells of the host plant. It seems possible that antihost antisera could be prepared which would contain antibodies specific for these enzymes; and that, as a result of serological reactions with these destructive enzymes, the virus could be free of their action.

The apparent resistance of tobacco mosaic virus to attack by proteolytic enzymes and ribonuclease may not be at all typical of the response of most plant viruses to enzymes released from disrupted plant cells.

For some purposes, such as recognition of some of the spheroidal viruses by means of the electron microscope, it may not be necessary to separate the virus from unreacted antibodies. The common obstacle in recognition of such viruses is the fact that juice from both healthy and infected plants contains many structures which overlap known virus particles in size and shape. It should be possible to recognize a number of spheroidal plant viruses against a background of the much smaller antibodies if the host substances are eliminated by serological reaction. It would greatly extend the usefulness of the electron microscope in plant virus diagnosis if such spheroidal viruses could be recognized without elaborate procedures.

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A SEED-BORNE BEAN VIRUS OF WIDE HOST RANGE

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SUMMARY

A seed-borne virus in beans produced symptoms similar to those of common bean mosiac (BV_1). The "new" virus, however, infected lima beans, cowpea var. Ramshorn, chickpea, tepary bean, and spinach, in addition to several varieties of beans.

Symptoms on beans included veinal necrosis of the inoculated primary leaves, plant stunting, leaf cupping, epinasty, and mottle. On Great Northern U.I. 31 and Red Mexican varieties U.I. 34, 35, and Pinto U.I. 111, only veinal necrosis was produced on the inoculated leaves. Of these varieties, the virus was recovered only from Red Mexican U.I. 35.

Topcrop, Sanilac, Idaho Bountiful, and Michelite were immune. The virus was inactivated in 10 minutes between 60-65°C. Its dilution end point was between 1:10,000 and 1:50,000 and it was infectious after aging *in vitro* for 72 hours, but not for 96 hours, at 17°C. It did not cross-protect against common and yellow bean mosaic viruses. Tentatively, it is named Western Bean Mosaic virus.

In 1957, Columbia Pinto bean (*Phaseolus vulgaris* L.) was released to bean growers in the Pacific Northwest. This bean, developed cooperatively by the U.S.D.A. and Washington State University, is resistant to bean viruses 1 and 1A (New York 15 strain of bean virus 1). Shortly after the release of this bean, considerable virus infection, with symptoms suggestive of those produced by bean virus 1, was noted by certification inspectors of the Washington State Department of Agriculture. Subsequent tests by these men and by Robertson (2) revealed that this virus was transmitted in 2-3% of the seed. This was confirmed later by the authors. The results of a study to determine the identity of the virus are reported here.

MATERIALS AND METHODS.—Experiments were conducted in a greenhouse at 20-25°C. Stringless Green Refugee was used as a virus source plant. Inoculations were made by the Carborundum abrasion method on primary leaves about three-fourths expanded. Latency of the virus in varieties and species which did not show symptoms was tested by inoculating Stringless Green Refugee plants with the uninoculated leaves of the symptomless plants. The virus was recovered from all varieties and species showing systemic symptoms after inoculation.

Symptoms and host range.—Among 17 bean varieties, 8 were susceptible to systemic infection and 4 to veinal necrosis only in the inoculated leaves. Columbia Pinto (Fig. 1-C), Great Northern U.I. 123, Early Pink, Blue Lake, Black Valentine, Dwarf Horticultural, and Improved Tendergreen developed mottle and cupping symptoms in the trifoliolate leaves similar to those produced by bean virus 1. Stringless Green Refugee (Fig. 1-A, 1-B_1) was severely stunted with epinasty and cupping of the trifoliolate leaves. All varieties showing systemic symptoms developed veinal necrosis and yellowing in the inoculated primary leaves.

Resistant varieties, showing only veinal necrosis and yellowing of the inoculated primary leaves, were Great Northern U.I. 31 (Fig. 1-D) and the Red Mexican varieties, U.I. 34 and 35, and Pinto U.I. 111. Topcrop, Idaho Refugee, Idaho Bountiful, Michelite, and Sanilac were immune.

Of the bean varieties not showing systemic symptoms, the virus was obtained in subinoculations to Stringless Green Refugee only from Red Mexican U.I. 35.

Four other species of legumes were susceptible to this virus. *Phaseolus lunatus* L. (lima bean var. Thorogreen) developed early vein clearing followed by stunting of the plant, some leaf cupping, and a very mild mottle (Fig. 1-B_2). On *P. acutifolius* Gray var. *latifolius* Freeman (tepary bean), the virus caused severe stunting, top necrosis, and leaf cupping. Vein clearing followed by veinal chlorosis and mottling occurred on *Vigna sinensis* (Torner) Savi (cowpea var. Ramshorn). *Cicer arietinum* L. (chickpea) showed a distinct mottling and stunting.

Two inoculation trials on spinach *Spinacia oleracea* L.) failed to produce symptoms other than slight stunting. In both instances, however, subinoculation from spinach to Stringless Green Refugee beans induced typical symptoms.

Plants found to be immune to the virus included *Crotalaria spectabilis* Roth (crotalaria), *Cyamopsis tetragonoloba* (L.) Taub (guar), *Glycine max* (L.) Merr. (soybean var. Biloxi and Lee), *Medicago sativa* L. (alfalfa var. Ranger), *Melilotus officinalis* (L.) Lam. (yellow sweet clover), *Pisum sativum* L. (pea var. Perfected Wales), *Stizolobium deeringianum* Bort. (velvet bean), *Trifolium incarnatum* L. (Crimson clover var. Dixie Reseeding), *T. pratense* L. (red clover var. Kenland), *T. repens* L. (ladino clover var. Pilgrim), *Vicia faba* L. (broadbean), *Vigna sinensis* L. (cowpea var. Blackeye), *Lathyrus odoratus* L. (sweetpea), *Cucumis sativus* L. (cucumber var. National Pickling), *Lycopersicon esculentum* Mill. (tomato var. Rutgers), *Solanum melongena* L. (eggplant var. Black Beauty), and *Nicotiana tabacum* L. (tobacco var. Samsun and Bottom Special).

Physical properties.—Expressed juice from virus-infected Stringless Green Refugee plants was used in these studies and results are based on systemic infection in this variety. The virus was inactivated in 10 minutes at 60-65°C. It withstood 1:10,000 dilution but not 1:50,000. Stored *in vitro* at 17°C, it was infectious after 72 hours, but not after 96 hours.

Cross-protection.—Experiments were conducted to determine if western bean mosaic virus was related to BV1 or BV2 (yellow bean mosaic virus). A known strain of BV1 was used to inoculate the primary leaves of Stringless Green Refugee plants. After the trifoliolate leaves showed mosaic symptoms, the primary leaves were inoculated with western bean mosaic virus. After 8-16 days, each infected plant was evaluated for the presence of the western mosaic virus by subinoculation from the trifoliolate leaves to BV1-immune Columbia Pinto plants. No cross-protection occurred: the western mosaic virus was transmitted as readily from the Stringless Green Refugee plants with the double inoculation as from control plants inoculated only with the western mosaic virus.

To determine whether the new virus is related to BV2, Stringless Green Refugee plants were inoculated with western mosaic virus followed by BV2 after symptoms of infection by the 1st virus were evident. BV2 infected most of these plants as shown by infection in subinoculated guar, Perfected Wales peas, and Biloxi soybeans, all of which are susceptible to BV2 but not

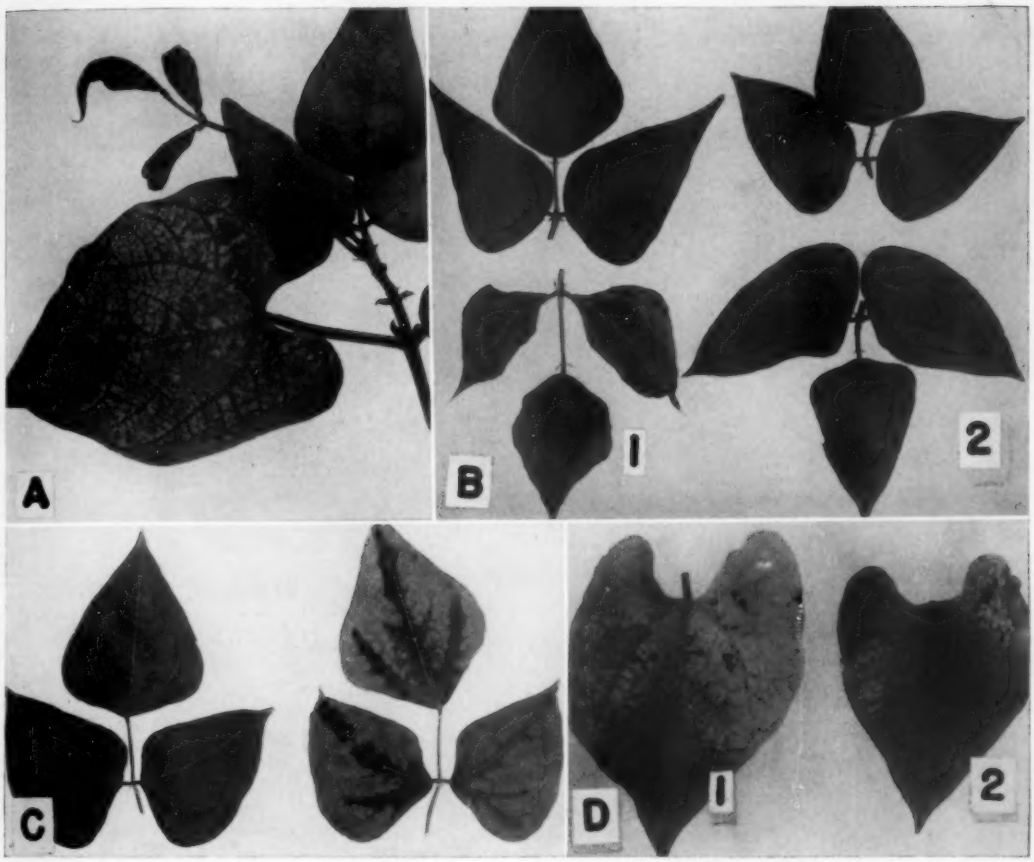

Fig. 1. A,B$_1$) Symptoms of western bean mosaic on Stringless Green Refugee. **B$_2$)** Vein clearing on Greenseeded Henderson bush lima bean. **C)** Symptoms on Columbia Pinto. **D)** The necrotic reaction of inoculated primaries on **1)** Great Northern 31 and **2)** Red Mexican U.I. 35.

to western mosaic virus. No cross-protection was observed in 4 tests.

Transmission.—Robertson (2) found that the new virus is transmitted by 3 species of aphids. It also is transmitted readily by leaf-abrasion methods. Further tests on seed transmission showed that the virus was transmitted in seed from greenhouse-grown and inoculated Stringless Green Refugee plants as well as in seed from field-infected Columbia Pinto. Seed from the latter source produced the same percentage of infected plants (2-3%) 3 years after they were harvested as they did immediately after harvest.

Discussion.—The properties of the western bean mosaic virus differ from those reported for any other virus-infecting beans. Its mosaic symptom expression and seed-borne nature suggest a close relationship to BV1 and its variant strains. In fact, bean varietal reactions to the western mosaic virus are very similar to those of a new seed-borne virus reported by Dean and Wilson (1) as a variant of BV1. The more stable physical properties and differing host range of the western mosaic virus, however, are evidence that it is unrelated to this group. Additional evidence is presented in the cross-protection tests. These tests, however, would not detect reduction in virus titer. It can only be said that *complete* cross-protection to either BV1 or BV2 was not evident.

Western bean mosaic virus also is different from BV2 in that it is seed-borne and does not infect BV2 susceptible peas, yellow sweet clover, and *Vicia fava*.

The unusual combination of veinal necrosis in inoculated primary leaves, in addition to the systemic mottle produced by the western mosaic virus, is similar to the symptom pattern described by Yerkes and Patino (4) for the severe bean mosaic virus. The latter virus, however, has greater resistance to physical tests and a wider host range than the western mosaic virus and is not seed-borne.

Physical properties of the western mosaic virus approach those of the cucumber mosaic and alfalfa mosaic viruses (3). On the other hand, the host range and seed-borne character of the western mosaic virus

set it apart from these viruses.

Considering the breadth of its host range, including at least 1 species outside of Leguminosae (spinach), no attempt is made on the basis of this study to assign a taxonomic position to the western mosaic virus. Further cross-protection and serological tests must be made.

The potential importance of the western bean mosaic virus is suggested by its seed-borne nature and its extensive occurrence in a 1957 planting of Columbia Pintos. During the past 2 growing seasons in which seed-borne viruses (BV1 and BV1a) have been seriously epiphytotic in Columbia Basin bean fields and in experimental plots, varieties susceptible to western bean mosaic only have remained largely free of mosaic symptoms. The estern bean mosaic virus has been identified only twice in naturally infected plants during the past 2 years. This indicates that the western bean mosaic virus with its low rate of seed transmission may be much less of a hazard than the common mosaic viruses.

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EFFECT OF VARIATION IN THE BACTERIAL SPOT PATHOGEN OF PEPPER AND TOMATO ON CONTROL WITH STREPTOMYCIN

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SUMMARY

Xanthomonas vesicatoria (Doidge) Dows isolates were obtained from both pepper and tomato in south Florida. Laboratory work found variation in colony type, physiology, and susceptibility to streptomycin. Four isolates, which differed in susceptibility to streptomycin in a disc-agar-plate test, were used to test control of bacterial spot in field plots by streptomycin sprays. Isolates A and F, which were relatively susceptible to streptomycin in plate tests, were controlled by streptomycin sprays, whereas isolates D and E, which were less susceptible to streptomycin in plate tests were not controlled by streptomycin sprays.

Bacterial spot, caused by *Xanthomonas vesicatoria* (Doidge) Dows., has become 1 of the most serious diseases of tomatoes and peppers in Florida. Weber and Ramsey (14) reported the disease on tomatoes in 1926 and Weber (13) on peppers in 1932. In the latter publication, seed treatment with mercuric chloride, spray applications with bordeaux mixture, and crop rotation were recommended for control. Later basic coppers were substituted for bordeaux in the control program. These recommendations at best prove adequate only under conditions of mild disease development. Under severe disease conditions, Ruehle (11) found that basic coppers did not control bacterial spot on tomatoes and that the use of bordeaux mixture resulted in increased numbers of diseased fruit.

A significant development in control of bacterial spot came with the advent of streptomycin early in the past decade. Since then there have been numerous field trials with the material in south Florida. Results have been variable. From 1953 to 1956 Conover (2, 3) obtained good control of bacterial spot of tomato with streptomycin. For example, in 1955 he reported a 74% reduction in number of diseased tomato fruits on plants sprayed with streptomycin at 100 ppm. In 1957, however, Conover (4) found that streptomycin spray resulted in poor control. Comparisons of streptomycin with basic copper have been indicative of the variability. In 1954, Cox (5) obtained equal control of bacterial spot on pepper with either streptomycin at 100 ppm or basic copper. Stall (12) reported the same relationship for control of spot on tomato in 1959. In 1957, Cox and VanNostran (7) found streptomycin at 100 ppm gave better control of this disease on pepper than basic copper; but that same year, strepto-

TABLE 1. Some characteristics of the bacterial isolates compared with those listed for *Xanthomonas vesicatoria* in Bergey's Manual

Isolate	Source	Colony surface	Flagella	Gelatin liquification	Sugar[a] solution acidified	Casein precipitated	Fat digested	Nitrite produced from nitrate	Hydrogen sulfide produced
A	Tomato	Smooth	1 polar	+	+	+	+	+	+
D	Pepper	Smooth	1 polar	+	+	+	−	−	+
E	Pepper	Rugose	1 polar	−	−	−	+	−	+
F	Pepper	Smooth	Mixture[b]	+	+	−	−	−	−
X. vesicatoria		Smooth	1 polar	+	+	+	+	−	+

[a] Sugars used were glucose, lactose, and sucrose.
[b] Early in the investigation, cells in this isolate had peritrichous flagella; later, after isolation from inoculated plants, only single polar flagella could be found.

mycin at 200 ppm was inferior to basic copper for control on tomato (6). Work reported here was undertaken to determine the importance of variability of the causal organism on control of bacterial spot with streptomycin.

Selection and characterization of isolates.—In the spring of 1959, over 600 mass isolates of the bacterial spot organism taken from tomato and pepper in Broward, St. Lucie, and Palm Beach counties were obtained by plating diseased leaf sections on nutrient agar. An attempt was made to obtain pure isolates from 50 of these by means of dilution series and selection of single colonies. Representative isolates of the most common cultural types, which remained macroscopically unchanged during a month in culture, were selected for experimentation and given letter designations. On the basis of laboratory data, isolates A, D, E, and F were selected for study in field plots. Results with these 4 isolates are presented.

All 4 of the isolates were pathogenic on pepper in greenhouse inoculation tests, produced a yellow pigment on nutrient agar, and were gram negative rods. These characteristics were considered sufficient evidence to classify isolates as *X. vesicatoria.*

Some of the characteristics of these isolates compared with those listed for *X. vesicatoria* in the 7th edition of Bergey's Manual (1) are given in Table 1. About 33% of the original isolates were of the rugose colony type listed for isolate E. These could not be distinguished from the smooth colony type in pathogenicity or symptom expression on pepper. The isolates did not react to all physiological tests as indicated for *X. vesicatoria* in Bergey's Manual. These differences are not presented as having taxonomic significance, since no thorough study of each reaction was undertaken. Although all the tests were repeated at least twice with the same results, only 1 method was used for each test. For example, stab cultures in 13% gelatin were used to test gelatin liquification. If more dilute concentrations of gelatin, or streak cultures in petri plates had been used, isolate E might have given a positive test. The characteristics presented in Table 1, however, are indicative of a highly variable population of *X. vesicatoria* in south Florida.

Susceptibility to streptomycin in laboratory tests.—Susceptibility of the isolates to streptomycin was checked using a disc-agar-plate method. The test was performed 2 times; with Agri-mycin 100 (15% streptomycin as sulfate plus 1.5% Terramycin); and next with Phytomycin (20% streptomycin as nitrate). In this method, 7-mm blotter paper discs were soaked for 1 hour or more in streptomycin solutions at concentrations of 12, 25, 50, and 100 ppm of active material. The discs were removed from the streptomycin solutions, placed on paper towels momentarily to remove excess moisture, then placed on nutrient agar in petri plates. The agar plates were atomized with a bacterial suspension standardized to 50% light transmission using a Bausch and Lomb colorimeter set at 50-mμ wavelength. After a 24-hour incubation period at 30°C, width of the bacterial inhibition zones was measured. Two plates with 5 discs each were used for each concentration of streptomycin. Average widths of inhibitory zones (Table 2) show that the isolates also were variable in susceptibility to streptomycin.

Susceptibility to streptomycin in field tests.—Pepper plants were inoculated with the isolates in field plots to test the relationship of laboratory results to field control. A 4 x 4 Latin square design with split plots was used. Main plot treatments were the bacterial isolates and subplots were either sprayed with Agrimycin 100 or unsprayed. Each main plot consisted of two 30-ft rows of peppers which were divided in half to form subplots. Plots were 60 ft apart and were surrounded by 2 rows of sunflowers to inhibit spread

TABLE 2. Width of zones of inhibition of bacterial spot isolates measured from the edge of paper discs soaked in streptomycin

	Width of inhibitory zones in mm							
	Streptomycin sulfate[a] (ppm)				Streptomycin nitrate[b] (ppm)			
Isolate	12	25	50	100	12	25	50	100
A	5	6	7	8	7	7	8	9
D	4	5	6	6	3	3	4	4
E	2	2	3	4	0	0	1	2
F	3	4	6	7	3	5	7	8

[a] From Agri-mycin 100; active materials are streptomycin as sulfate 15% plus Terramycin 1.5%.
[b] From Phytomycin; active material is streptomycin as nitrate 20%.

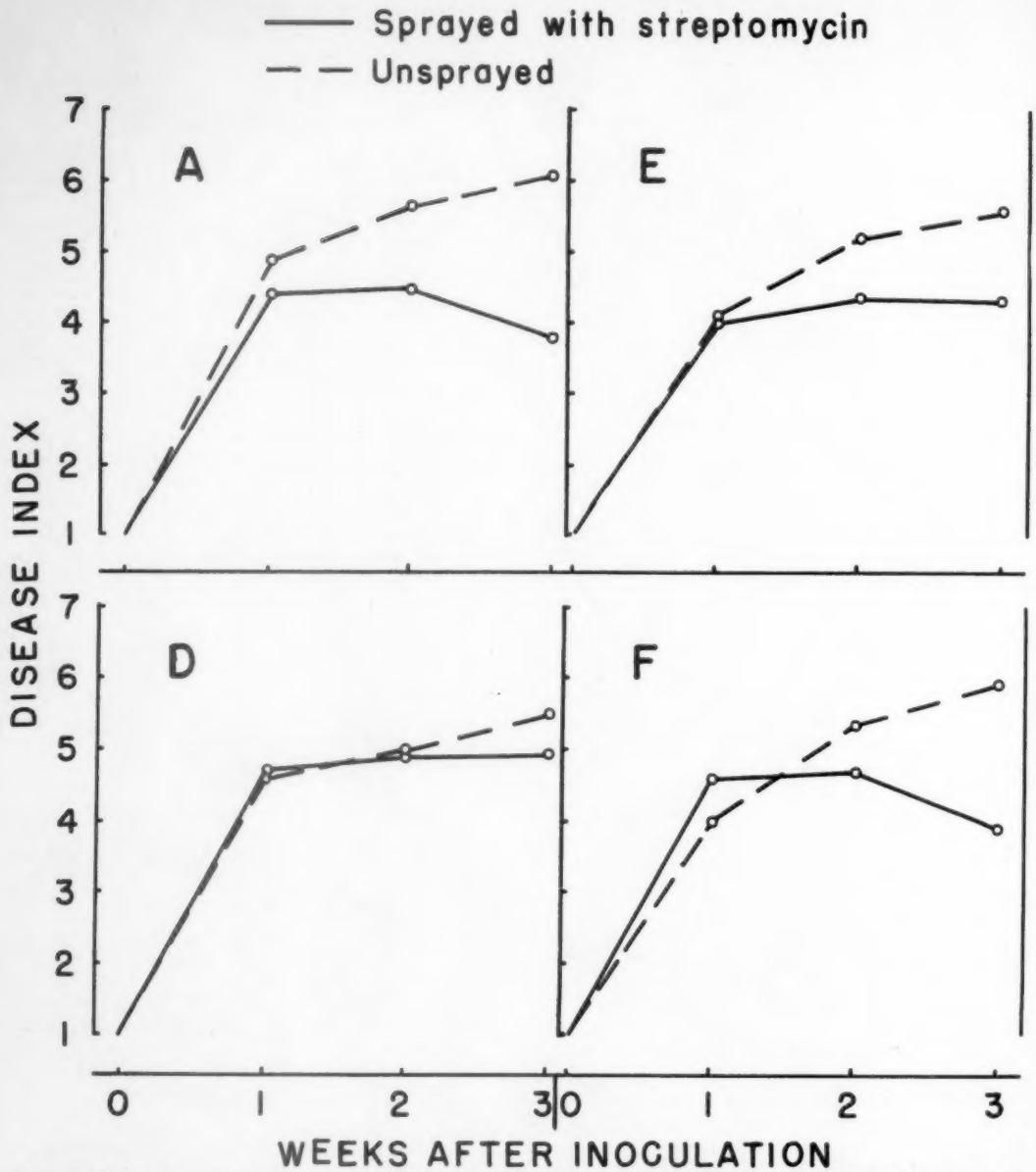

Fig. 1. Bacterial spot development on pepper plants in field plots inoculated with isolates **A, D, E,** and **F** and sprayed with streptomycin. Spray applications reduced disease development with isolates A and F, which had wider inhibition zones in disc-agar-plate tests. The third-week "t" values for sprayed *vs.* unsprayed plots were: A = 6.7, D = 0.8, E = 1.5, and F = 6.9. Values necessary for significance at 0.05 and 0.01 levels are 2.4 and 3.7. Disease rating system was 1 = no disease and 12 = dead plants.

of the bacterial isolates from 1 plot to the next. Pepper seed was sown the last week in September and the plants were maintained virtually bacterial-spot free until inoculation. Those plots receiving Agri-mycin were sprayed with 100 ppm on December 29, 30, and 31. On the evening of December 31, the pepper plants were sprayed with the bacterial suspensions, using a hand sprayer. Disease ratings were made 1 week later, using a scale of 1-12, according to the Horsfall-Barratt system (10). No difference could be detected between sprayed and unsprayed plants inoculated with any of the bacterial isolates.

Since Agri-mycin 100 at 100 ppm did not hinder disease development, the concentration was increased to 500 ppm. Three applications were made to the same plots on January 8, 13, and 18. Additional disease ratings were taken 2 and 3 weeks after inoculation. Disease development over the 3-week period is presented in Fig. 1. At the 2nd week after inoculation, there were still no differences. At the 3rd week, differences between sprayed and unsprayed plants occurred in plots inoculated with isolates A and F, but not in plots inoculated with isolates D and E. Using the "t" test as a measure of significance between sprayed and unsprayed plants, values for isolates A and F at the 3rd week were 6.7 and 6.9, respectively. A value of 3.7 is necessary for significance at the 1% level of probability. These results agree with the relative susceptibility of the isolates to streptomycin in disc-agar-plate tests, where isolates A and F had the wider inhibition zones. No significant difference could be detected in pathogenicity of the 4 isolates in unsprayed plots.

A final check was made on the purity of the bacterial isolates by isolations from leaf lesions in each plot following the last disease rating. Eighty-five to 94% of the isolates had the same colony and morphological characteristics as the source of inoculum. This would indicate that little field spread occurred. Also in support of this hypothesis, weather conditions were favorable for disease development, but not dissemination of the organism; *i.e.*, no rainfall and heavy dews every night. One notable change occurred in isolate F. Peritrichous flagella were observed in the original culture, but only single polar flagella could be found on cells cultured from field plots. This would indicate that isolate F was a mixture of cell types (Table 1).

Discussion.—In this work, differences in susceptibility of isolates to streptomycin were demonstrated and these differences were found to be significant in field control of bacterial spot with streptomycin. Workers (2, 12) have speculated that control of bacterial spot by streptomycin is dependent on factors affecting the absorption of streptomycin, the severity of the disease, and spraying techniques. Undoubtedly, the importance of these factors cannot be minimized; but, in many instances they cannot logically explain poor control. Results of these tests indicate that variation in the pathogen may be another factor contributing to inconsistencies in field control of bacterial spot with streptomycin.

The isolates used in this work are representative of the cultural types frequently isolated from diseased tissue; however, the small sample tested probably does not represent the possible range. Cultural and physiological characteristics of these isolates are indicative of a high degree of variability in the bacterial spot pathogen in south Florida. Considering this variability, along with capricious control of bacterial spot in field plots, and medical experience with resistance to antibiotics, it was not surprising that variation also occurred in susceptibility to streptomycin.

English and VanHalsema (9) found that a streptomycin-resistant population of *X. vesicatoria* developed in culture upon exposure to increasing antibiotic concentrations. Whether the widespread use of streptomycin for bacterial spot in south Florida has had a selective effect on the population of bacteria is not known. Early in the evaluation of streptomycin as a control for the disease, however, reports of poor control can be found. For example, Darby (8) saw very little control in plot work in 1954. Such reports may mean that populations of resistant strains have been present all along. The streptomycin-resistant portion of the population may increase in relation to the streptomycin-susceptible portion: this should be investigated.

This work places some restrictions on recommendations of streptomycin for control of bacterial spot of tomato and pepper. In some cases, recommended dosages of streptomycin would not be adequate, whereas in other cases excellent control would be obtained. The degree of control would probably differ, from area to area or field to field, depending upon the susceptibility to streptomycin of the predominant strains of bacteria present.

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RELATION OF TEMPERATURE TO SPORE GERMINATION, GROWTH, AND DISEASE DEVELOPMENT BY GILBERTELLA PERSICARIA

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SUMMARY

The temperature range for sporangiospore germination, mycelial growth in culture, and fruit rot was 4-39°C, the optimum being 30-33°C. No apparent differences in mycelial growth *in vitro* or fruit-rot production existed among 7 isolates. *Gilbertella persicaria* behaved similarly to *Rhizopus stolonifer* in these temperature relationships, except for the shorter time required for sporangiospore germination. A very close correlation existed between the minimum, optimum, and maximum temperature requirements for germination of spores, growth rate, and disease production by *G. persicaria*. The characteristic growth and sporulation occurred on all inoculated stone fruits, strawberry fruit, and fleshy roots of sweet potato.

INTRODUCTION.—The pathogenicity of *Gilbertella persicaria* (Eddy) Hesseltine on ripe peaches (*Prunus persica* Batsch.) was established by both Eddy (2) and Hesseltine (4). In both the Sacramento and San Joaquin valleys of California, *G. persicaria* was isolated from stone-fruit species in 1958 (5). In this paper, results are reported of a more complete study on the temperature requirements for spore germination and mycelial growth *in vitro* and disease development particularly on peach and other stone fruits. This information on temperature relationships is compared with that of *Rhizopus stolonifer* (Erh. ex Fr.) Vuillemin isolates from peach.

MATERIALS AND METHODS.—The isolates used were characterized by Butler, *et al.* (1). Effects of temperature on fruit-rot production and mycelial growth in culture were studied with 7 isolates, obtained from different areas of California, from fruits of peach (*P. persica*) (MP-4), nectarine (*P. persica* var. *nectarina* Maxim.), Japanese plum (*P. salicina* Lindl.), apricot (*P. armeniaca* L.), and tomato (*Lycopersicon esculentum* Mill.). Temperatures used in these tests were 4°, 12°, 15°, 18°, 21°, 24°, 27°, 30°, 33°, 36°, and 39°C, unless otherwise indicated. Germination studies were made with sporangiospores taken from 3-5-day-old cultures on 2% potato-dextrose agar (PDA). Droplets of sporangiospore suspensions in redistilled water were placed on PDA and incubated for 4, 8, 12, and 24 hours. Spores were recorded as germinated when germ tubes were as long or longer than the width of the sporangiospore; 100 spores were examined on each of 3 replications. Sporangial production and colony growth tests were made on PDA and modified Mucor agar (3). To determine fruit rotting at various temperatures, 10 harvest-mature Late Champion peach fruits were inoculated by placing a drop of spore suspension in wounds (2-mm punctures). Ten inoculated fruits were placed in each plastic container and 1 container was held at each temperature for 72 hours. The peach pit was removed and the diseased and healthy portions were weighed. Inoculations with *R. stolonifer* isolate from peach were made in the same manner. Studies on the development of both diseases on other stone fruits, strawberry, and sweet potato were made only at 24°C.

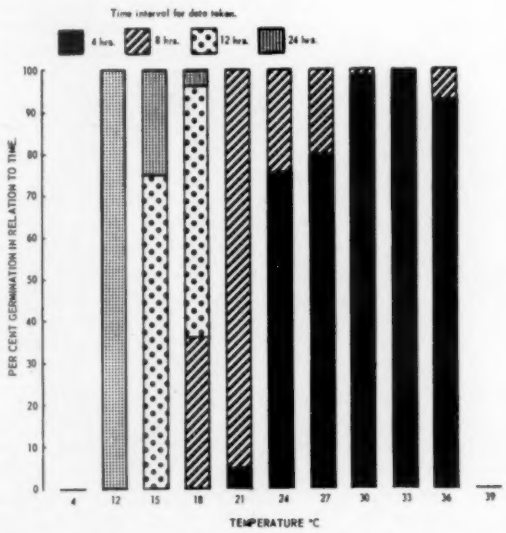

Fig. 1. Percentage of germination of *Gilbertella persicaria* (MP-4) sporangiospores on 2% potato-dextrose agar at various temperatures and time limits.

RESULTS.—*Causal organism.*—1) *Rate of sporangiospore germination.*— Fig. 1 shows the effects of time and temperature on sporangiospore germination of isolate MP-4 from peach. From 24-36°C, more than 75% of the sporangiospores germinated within 4 hours. Within 24 hours, 100% of the sporangiospores germinated at temperatures between 12° and 36°C; none germinated at 4° or 39°C. Transfers to 24° from 4° and 39°C resulted in normal germination. These sporangiospores swelled from an average of 6.4 x 9.8 μ to an almost spherical shape, with an average measurement of 14.2 μ at germination.

2) *Colony growth and sporangium production.*—At 18-39°C, a growth of 58 mm in diameter was required

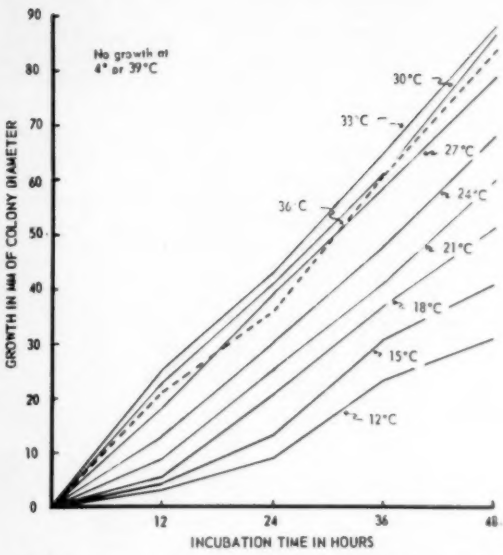

Fig. 2. Average growth of 7 isolates of *Gilbertella persicaria* at various temperatures and time limits on growth tubes with modified Hesseltine's medium. The dotted line (36°C) shows reduction in colony diameter from the optimum at 30-33°C.

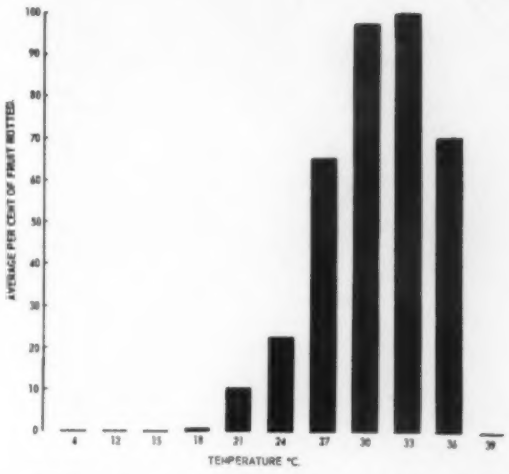

Fig. 3. Percentage of fruit rot of Late Champion peaches inoculated with *Gilbertella persicaria* (MP-4) sporangiospores and held at various temperatures for 72 hours.

for sporangial development on PDA in petri plates. At 15° and 36°C, a few sporangia developed on cultures 61 mm in diameter, after about 3 mm of aerial mycelium had developed. Continued exposure to 36°C resulted in only a few black sporangia in 9 days, although the fungus extended completely over the plate with a 3-4 mm layer of aerial mycelium in less than 72 hours. Lowering the temperature from 36° to 24°C resulted in little increase in sporangial production within 72 hours, and new sporangia always developed from new sporangiophores. Sporangia generally were initiated at the point of inoculation. Fig. 2 shows the average colony growth of 7 isolates on synthetic "Mucor agar" (3) in which 2 g of yeast extract were substituted for 0.5 mg of thiamine chloride. A 1-mm biscuit was cut from a heavy sporangiospore-laden agar medium and placed on the agar inside a dammed test tube (22 x 175 mm). Three replications of each of the 7 isolates were incubated at 4-39°C, and growth was measured at 12, 24, 36, and 48 hours. No growth occurred at 4° or 39°C. No significant differences were apparent among the 7 isolates at these temperatures. Growth was optimum at 33°C, but not significantly different from that at 30°C, and a slight reduction in colony diameter at 36°C is indicated by a dotted line.

Disease.—Fruit rot development.—Ten harvest-mature Late Champion peaches were inoculated with sporangiospore suspension (MP-4) and incubated 72 hours at 4-39°C in plastic containers with loose lids. Fig. 3 shows complete invasion of fruit in 72 hours at 30-33°C. No rot developed at 4-15° or 39°C. Isolate MP-4 sporulated most heavily at 30°C. At 36° and

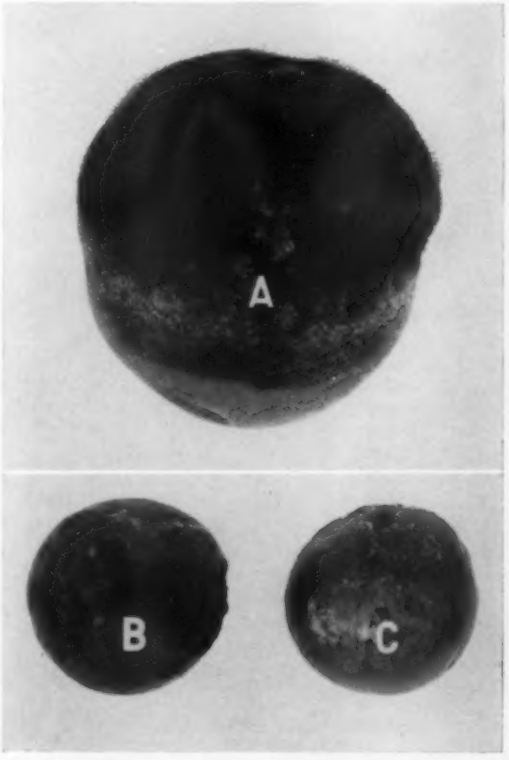

Fig. 4. Comparison of *Gilbertella persicaria* **A)** and *Rhizopus stolonifer* **B, C)** on peach fruits rotted under orchard conditions.

below 21°C, it sporulated only sparingly. Effects of temperature on 6 other isolates were studied at 4°, 30°, 33°, 36°, and 39°C. These percentages of fruit rot were similar to those given in Fig. 3. In a similar experiment, the fungus rotted 10 Tilton apricot fruits after 6 days at 12° and 15°C.

When inoculated with isolate MP-4 and incubated at 24°C in loosely covered plastic containers, typical sporulation and rot development were exhibited on the following: 1) *Ripe fruits*: apricot (*Prunus armeniaca*), cultivars Royal and Tilton; peach (*P. persica*), cultivars Regular Elberta, Fay Elberta, J. H. Hale, Babcock, Late Champion, Miller's Late, Palora, and Fortuna; nectarine (*P. persica* var. *nectarina*), cultivars Quetta and Sun Grand; cherry (*P. cerasus* L.), cultivar Montmorency; plum (*P. salicina*), cultivar Santa Rosa; and cultivated strawberry (*Fragaria* sp.). 2) *Green fruits*: peach, cultivars Walton, Halford, and Stuart; apricot, cultivar Royal; and almond (*P. amygdalus* Batsch.) cultivar Drake. 3) On fleshy roots of sweet potato [*Ipomoea batatas* (L.) Lam.] only tufts of sporulation occurred.

DISCUSSION.—*Gilbertella persicaria* rot on peach fruit is shown in Fig. 4-A. Under orchard environment, *R. stolonifer* produces a similar rot on peach (Fig. 4-B, C) in California. Production of short turf by *Rhizopus* under low relative humidity on strawberry has been reported by Stevens and Wilcox (6).

Although differences in the rate of sporangiospore germination were noted between *G. persicaria* and *R. stolonifer*, similar growth patterns were obtained for both organisms on PDA at temperature ranges between 4-39°C. The trends as observed for growth also were shown for both pathogens in their ability to produce rot at these temperatures.

In these studies in the laboratory, the appearance of *G. persicaria* could not be confused with that of *R. stolonifer*, although in some cases under field conditions they were very difficult to distinguish.

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REPORT AND ABSTRACTS OF THE 1960 MEETING OF THE NORTHEASTERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 20th Annual Meetings of the Northeastern Division of The American Phytopathological Society were held on November 3 and 4, 1960, in the auditorium of the Eastern States Farmers' Exchange, West Springfield, Mass. Approximately 200 were in attendance and 34 scientific papers were presented. An evening session included a most interesting discussion by Dr. J. S. Boyce on the early history of forest pathology.

With the increasing number of papers submitted for the divisional meeting, it was decided that as many of the following measures would be adopted as necessary in scheduling future programs.

1. Shorten presentation time to 10 minutes.
2. Limit each author to 1 paper.
3. Accept only papers that will be published in PHYTOPATHOLOGY.
4. And, if necessary, extend the length of the Session to include an additional half day.

A committee was appointed to study ways of selecting candidates for recognition by the Society for outstanding contributions in the field of Plant Pathology.

Officers selected to serve during the next year are as follows:

President	—D. H. Marsden
Vice-President	—R. H. Daines
Secretary-Treasurer	—W. F. Mai
Councilor	—G. A. Brandes

CONTROL OF TOBACCO FLECK BY DUSTING WITH ZINEB—P. J. Anderson—Dithane (65% zineb) was applied to shade tobacco fields every 5 days at the rate of 16 lb to the acre. A severe outbreak of fleck which came the next day after the first dust application was reduced by only about 50%; but later, less severe outbreaks were completely prevented by this program, while 67% of the plants on the fields not dusted were damaged by fleck.

SYNERGISM BETWEEN CUCUMBER MOSAIC VIRUS AND RHIZOCTONIA IN RELATION TO RHIZOCTONIA DAMPING-OFF OF CUCUMBER—D. F. Bateman—Post-emergence damping-off caused by *Rhizoctonia* sp. in cucumber (*Cucumis sativus* L.) was increased from 10-15% to 60-87%, by cucumber mosaic virus (CMV) infection. The final results were similar whether the *Rhizoctonia* inoculum was added to the soil at the start of the experiment or at

the time the cucumber cotyledons were mechanically inoculated with CMV; however, greatest synergistic activity between the pathogens occurred when a small quantity of *Rhizoctonia* inoculum and a highly infectious CMV inoculum were employed. Differences in the incidence of stem attack by *Rhizoctonia* among plants inoculated with *Rhizoctonia* and among those inoculated with both pathogens were generally apparent on the 3rd or 4th day after inoculation with CMV. Damping-off data were recorded 7 days after inoculation with CMV. The rapidity with which damping-off occurred in the doubly inoculated plants suggested that movement of materials from the roots to the CMV-infected cotyledons, which showed increased respiration 48 hours after inoculation, increases the susceptibility of fungus attack.

WILT RESISTANCE MECHANISMS IN GROS MICHEL BANANA—C. H. Beckman, S. Halmos, and M. E. Mace—Resistance to *Fusarium* wilt appears dependent upon vascular perforation plates which delay the advance of *Fusarium oxysporum* until host responses become operative. Wounded roots inoculated with microspores were examined microscopically at daily intervals. Response sites were located by red vinyl particles mixed with spores. Penetration of perforation plates occurred in 1 day. Hyphae grew 0.5 mm per day thereafter and sporulated after 3 days. Ruthenium staining gel was visible at 1 day and filled vessels for several mm by 3 days. Small tyloses were present at 4 days and filled vessels for 7 mm (avg.) beyond spore trapping sites at 5 days and to 33 mm at 10 days. These responses occurred with rhizoplane microflora or *Fusarium* alone, but not without microorganisms. Results suggest that 1) under conditions used, gel and tyloses prevent systemic invasion after initial spore trapping; 2) gel and tyloses formation are host-protective responses induced by many microorganisms; 3) once within the host, pathogenicity of *Fusarium* depends upon its ability to precede host-protective responses under certain environmental conditions, rather than upon an unusual capacity to induce vascular occlusion; and 4) given systemic distribution of *Fusarium* these localized, protective responses become general, lethal responses.

SEROLOGICAL SEPARATION OF FORMS AND PHYSIOLOGIC RACES OF PATHOGENIC FUSARIUM OXYSPORUM—E. W. Buxton, W. Culbreth, and R. G. Esposito—Neither *formae speciales* nor physiologic races of *Fusarium oxysporum* can be distinguished from each other in culture; differentiation is based on specific pathogenicity. To find an *in vitro* method for separating isolates, antisera were obtained by injecting rabbits with spores disintegrated by alternate freezing and thawing. Agglutination tests with antisera to *F. oxysporum* f. *cubense* (cause of banana wilt) and *F. oxysporum* f. *pisi* (cause of pea wilt) with 23 *formae* and races as antigens gave a relatively simple means of *in vitro* identification. Using several dilutions of untreated antigens and antisera, it was possible to characterize races of *F. o. cubense* and *F. o. pisi*. For example, race 2 of *F. o. pisi* did not agglutinate with antiserum to race 1, nor did race 2 of *F. o. cubense* react with antiserum to its race 1. Absorption of antisera with several antigens enabled further differentiation, and *F. o. lycopersici* was distinguished from both *F. o. cubense* race 1 and *F. o. pisi* race 1. Agar gel-diffusion plates, testing antigens against antisera, also corroborated these results. In addition to the presence or absence of interactions for identification, several distinct interaction lines between some combinations indicated that certain *formae* contain a number of different antigens.

A SIMPLIFIED METHOD FOR CULTURE OF CERATOCYSTIS ULMI—R. J. Campana and M. A. Rosinski—Septic culture of the Dutch elm disease fungus, utilizing a moist chamber technique, was tested with success. Infected and discolored elm discs about ½ in. long and ½ in. in diameter were rinsed in tap water, drained, and stoppered in vials. More than 1,400 samples of infected wood yielded coremia of *Ceratocystis ulmi* in presence of contaminating fungi or bacteria. Of 440 paired samples from 220 infected trees, comparing aseptic-plate with septic-vial culture, the fungus was confirmed in 46 cases by vial but not by plate. In 1 comparison it was confirmed by plate but not by vial. Coremia developed more reliably in wood immersed and drained, than in wood not soaked or with free water. Coremia developed within 3-14 days. In mixed suspensions with species of *Verticillium, Fusarium, Penicillium, Trichoderma, Aspergillus, Rhizopus, Alternaria,* or elm-inhabiting bacteria, *C. ulmi* developed coremia readily on inoculated elm discs. Suspensions of the same fungi and bacteria deposited on elm discs from diseased trees did not prevent development of coremia. To confirm identification of *C. ulmi*, coremia were streaked on PDA. *C. ulmi* was neither obscured by nor indistinct from any organisms with which its coremia could be confused.

REDUCTION OF POST-HARVEST DECAY IN RASPBERRIES WITH SO_2—R. A. Cappellini, A. W. Stretch, and G. S. Walton—Latham raspberries were exposed to single and multiple doses of sulfur dioxide (SO_2) at approximately 80°F. Concentrations of 0.13, 0.25, and 0.50% SO_2, delivered at 20 psi and regulated by a flow-meter, were blower circulated throughout a fumigation chamber during a 20-minute period. The initial fumigation was made approximately 4 hours after harvest, additional fumigations were either 24, 48, or 72 hours later. Data on "visible mold" were taken after the berries were stored for 2-7 days at 50°, 70°, and room temperatures (80-85°F.). *Botrytis* sp. and *Cladosporium* sp. were the predominant fungi occurring on the berries in these tests. Highly significant reduction in decay was obtained with all treatments at all storage temperatures. A softening and slight bleaching of the berries were noted with the 0.50% SO_2 treatment, especially those stored at the higher temperature (80-85°F.), however, some color deterioration of the untreated berries was also noted at this temperature. With the lower concentrations (0.13 and 0.25%), a delayed effect on ripening was observed but is not considered injurious. Off-flavors were not detected in the treated berries.

APPLICATION OF SOIL FUNGICIDES FOR THE CONTROL OF DAMPING-OFF OF SPINACH—R. C. Cetas—The application of captan, dichlone, maneb, nabam, and Phaltan (50% N-trichloromethylthiophthalimide) to the seed furrow resulted in satisfactory control of damping-off of spinach (*Spinacia oleracea* L. var. America) caused by the *Fusarium-Pythium-Rhizoctonia* complex in 12 field experiments in 1960. Satisfactory stands resulted when the fungicides were applied to the seed furrow in 30 gal of water per acre (34,400 ft of row). In contrast, band application of the fungicides to the surface of the seeded row as sprays in 100 gal of water per acre failed to improve stands substantially. Spraying half the fungicide dosage into the seed furrow and the remainder in a band on the surface of the seeded row was slightly less effective than spraying the full dosage into the seed furrow. When equal dosages of the fungicides were sprayed into the seed furrow, slightly greater initial stands resulted from the use of 30 gal of water per acre than from the use of 15 gal. The application of 25-30 lb of 10 and 20% active captan, maneb, or Phaltan dusts to the seed furrow was as effective as application of the same dosages as sprays.

SOIL FUNGICIDES FOR THE CONTROL OF DAMPING-OFF OF SPINACH—R. C. Cetas—In 1960 isolations were made from 221 diseased spinach (*Spinacia oleracea* L. var. America) seedlings and *Fusarium sp.* was isolated from 92 seedlings, *Pythium sp.* from 59, and *Rhizoctonia sp.* from 6. *Fusarium* and *Phythium* were isolated in a ratio of 1:1 in May and 2:1 in June and July. Fungicides were applied to the seed furrow as sprays in 15, 22, and 30 gal of water per acre (34,400 ft of row) and as dusts at the rate of 25-30 lb per acre. Under conditions favorable

for development of damping-off, initial stands commonly increased between 50 and 200%, and final stands and yields increased substantially. Initial stands increased an average of 53.3% in the 12 field experiments. The following fungicides and dosages per acre were equally effective: 6 and 8 quarts of 22% nabam; 1 lb active dichlone; and 2.5, 3.75, and 5.0 lb active of each of the following: captan, maneb, and Phaltan (50% N-trichloromethylthiophthalimide). Zineb, PCNB (pentachloronitrobenzene), and Miller 658 Fungicide (95% copper zinc chromate complex) were less effective. Captan-PCNB, captan-PCNB-maneb, and captan-PCNB-zineb mixtures were no more effective than captan or maneb alone.

PHYSIOLOGICAL ASPECTS OF CAPTAN AS A SPRAY CHEMICAL—G. R. Cooper, A. S. Allen, M. T. Hilborn, and R. C. McCrum—For several years it has been evident that some organic fungicides have effects on plants other than disease control. Physiological side effects, such as increased chlorophyll content and higher yields, have been noted. To investigate this problem more fully, duckweed was chosen as a laboratory test plant since it can be grown asexually as a clone in sterile nutrient solution. Two genera of duckweed were used, *Spirodela polyrhiza* (L.) Schleid., and *Lemma minor*, L.; both were sterilized by immersion in a weak sodium hypochlorite solution. Then selected plants were allowed to increase to provide clones of a sufficient size to complete the work. The duckweed was grown at 25°C under artificial light in Erlenmeyer flasks containing Hoagland's solution diluted 9:1 with water. Captan was added at various concentrations up to 25 ppm. Light intensity at the plant surface was 310 ft-c. Measurements included fresh wt, dry wt, increase in number of fronds per frond in the inoculation, and a determination of the CO_2 utilization rate by infrared gas analysis. Increases in all measurements were registered by the captan-treated plants when compared with the untreated control plants. The infrared gas analysis for the CO_2 content of the air above the plants indicated an interesting suppression of apparent photosynthesis by captan for a short period of time. This supression was followed by a marked increase in the rate of apparent photosynthesis.

INSECT TRANSMISSION OF CANKER STAIN FUNGUS, CERATOCYSTIS FIMBRIATA F. PLATANI—L. J. Crone and S. Bachelder—Insect transmission studies of *Ceratocystis fimbriata* f. *platani*, canker stain pathogen of *Platanus* sp., were conducted during August, 1959, and the summer of 1960. Coleopterous insects (principally Nitidulids) were hand-collected with disinfested instruments from diseased and healthy London plane trees in southern New Jersey. Some of the beetles were placed on a culture of the pathogen for ½ hour before caging them on healthy trees; others were transferred directly from diseased to healthy trees. When placed on a culture of the pathogen, many of the beetles fed upon the spores and mycelium. Ascospores adhered to many parts of their bodies. The beetles were caged over fresh knife-wounds on 3- and 4-year-old *P. acerifolia* at New Brunswick, N. J. for 1 to 2 weeks. Isolations were made approximately 1 month later from symptomatic trees. Transmission from cultures to healthy trees was obtained in 6 of 11 trials (1 to 4 beetles per trial) and from diseased trees to healthy trees in 2 of 7 trials. *Colastus unicolor*, *C. morio*, and *Carpophilus lugubris* transmitted the fungus from cultures; *Cryptarcha ampla* and *Laemophloeus biguttatus* transmitted the pathogen from diseased trees to healthy trees.

DODINE (CYPREX) EFFECTIVE AGAINST BACTERIAL SPOT OF PEACH—Robert H. Daines—Highly significant protection against bacterial spot (*Xanthomonas pruni*) on Sunhigh peaches was obtained in well-replicated plots, with ¼, ½, and 1 lb of dodine per 100 gal, alone, or with 1 lb of captan compared with a sulfur treatment. Control at 1 lb was highly significant compared with the zinc sulfate-lime treatment, a standard since its introduction in 1929 by Roberts. Injury to fruit was observed but no visible injury occurred on foliage. On trees sprayed with 1 lb of dodine, green areas were observed on about 1% of the immature fruit, however, they were somewhat less numerous on dodine-captan sprayed trees. By harvest time, these green areas had failed to develop a red color, resulting in yellow spots that were about ¼ in. in diameter, except at the apical end of the peach where they were somewhat larger. Such spots rarely occurred on fruits sprayed with ½ lb of dodine.

THE EFFECT OF QUACK GRASS AND BUCKWHEAT ON THE GROWTH OF TOMATO AND CORN PLANTS—Dean A. Eggert and Avery E. Rich—Tomato (*Lycopersicon esculentum*) and corn (*Zea mays*) plants were grown in pots of pasteurized soil to which had been added macerated leaves, stems, roots, rhizomes, or spikes of quack grass (*Agropyron repens*). Growth measurements indicated that the stems of quack grass were most toxic to both tomatoes and corn, followed by roots, rhizomes, and spikes. The macerated leaves stunted tomatoes slightly but did not stunt corn. Based on green and dry weights of tomato plants, quack grass stems were the most toxic, followed by roots, spikes, and rhizomes. Tomato and corn plants grown in soil to which macerated leaves had been added weighed more than comparable controls. Based on green and dry weights of corn, quack grass stems were most toxic, followed by rhizomes, roots, and spikes. The addition of macerated roots, stems, or leaves of buckwheat (*Fagopyrum sagittatum*) to the soil had little if any effect on the growth of tomato or corn plants.

THE MECHANISM OF ACTION OF COPPER-8-HYDROXYQUINOLATE ON FUNGAL SPORES—R. G. Esposito and C. H. Beckman—Studies of the mechanism of inhibition of the germination of microspores of *Fusarium oxysporum* by copper-8-hydroxyquinolate (copper-oxine) indicate a specific site of action. Pterins, related pterinoids, and their possible precursors have been tested for antagonism of the inhibition. Certain of these compounds are active antagonists of copper-oxine. Spectrophotometric studies indicate that the antagonism takes place intracellularly. The results of studies of synergy between copper-oxine and pterin analogue suggest identical sites of action. The data obtained support a mechanism of copper-oxine action based on interference with pterin biosynthesis and the metabolism of compounds with chelating groups of similar structure.

THE INTERACTION OF POTATO VIRUSES X AND Y IN INOCULATED TOBACCO LEAVES AT VARIOUS TEMPERATURES—R. E. Ford and A. Frank Ross—Young plants of *Nicotiana tobacum* L. var. Samsun NN, inoculated either with a mild strain of potato virus X (PVX) alone or with a mixture of PVX and potato virus Y (PVY), were maintained at 20°, 25°, or 30°C in constant-temperature chambers with about 600 ft-c of light 17 hours a day. Inoculated leaves were sampled at 5-day intervals, and the PVX activity of each expressed juice was compared with that of a frozen standard on *Gomphrena globosa* L. by 2-dilution assay. PVX concentration increased little or none after 10 days except possibly in doubly infected leaves at 25°C. In leaves inoculated with PVX alone, the virus level reached was lowest at 30°C and about the same at 20° and 25°C; in doubly infected leaves, PVX concentration was lowest at 30°, intermediate at 20°, and highest at 25°C. The ratio of PVX concentration (average of 10-, 15-, and 20-day samples) in doubly infected leaves to that in singly infected ones was 2.2 at 20°, 3.7 at 25°, and 4.7 at 30°C. In doubly infected leaves, the excess PVX synthesized as a consequence of PVY infection was about twice as great at 25°C as at 20° or 30°C.

ASSOCIATION OF DECLINE OF SPY 227 ROOTSTOCKS WITH CHLOROTIC LEAF SPOT VIRUS—R. M. Gilmer and K. D. Brase—Of 20 commercial apple vari-

eties propagated on Spy 227 budlings in 1958, 11 grew vigorously, 1 grew poorly, and 8 either failed completely or were in advanced decline by autumn, 1960. When indexed on Russian seedling R-12740-7A and *Malus platycarpa* (Long Ashton strain) in the winter of 1959-60, all 8 varieties that failed to grow or developed pronounced decline on Spy 227 were infected with chlorotic leaf spot virus (CLSV). Ten of 11 varieties that grew vigorously on Spy 227 were free of CLSV; inconclusive evidence was obtained that the remaining variety became infected with CLSV between propagation on Spy 227 in 1958 and indexing in 1959-60. Differences among varieties in rate of decline on Spy 227 and the occurrence of a single CLSV-free variety that grows weakly but does not decline on Spy 227 may indicate that more than 1 virus is involved. CLSV induces line pattern symptoms in *M. platycarpa*, and of the 2 names currently proposed for this virus, CLSV holds priority over the synonym *platycarpa* line pattern virus.

THE INFLUENCE OF PH AND CERTAIN PHOSPHORYLATED COMPOUNDS ON GERMINATION OF SPORES OF ASPERGILLUS NIGER IN MAGNESIUM-DEFICIENT MEDIA—Garold F. Gregory and J. Stanley Melching—Conidia of *Aspergillus niger* failed to germinate in a chemically defined medium (pH 4) lacking Mg and/or D-glucose but containing all other essential inorganic ions. Germination occurred in the minus-Mg medium when the pH was raised to 5.8 by addition of NaOH, $Ba(OH)_2$, or KOH. Within the range pH 5.8 to 6.6, the percentage germination was proportional to the hydroxyl ion concentration. Delayed germination, followed by a slight amount of submerged growth, occurred at pH 4 when Fe also was excluded from the minus-Mg medium. Germination appeared normal when D-glucose was replaced in the minus-Mg medium by fructose-1,6-diphosphate, fructose-6-phosphate, glucose-1-phosphate, or phosphoglycerate, although the subsequent growth appeared normal only with fructose-6-phosphate. When acid hydrolyzates or ashed residues of these phosphorylated compounds were added to minus-Mg medium containing D-glucose (pH 3.6), erratic and generally greatly delayed germination occurred. These results suggest that the germination induced by phosphorylated compounds is due only in part to mineral contaminants.

RESISTANCE TO SEVERAL VIRAL DISEASES INHERITED AS A UNIT—Francis O. Holmes—A line of tobacco that had been found to breed true with respect to an increased resistance to infection by tobacco-mosaic virus, as a result of earlier crossing with T.I. 245 tobacco, was studied further. Inoculation with 6 additional viruses showed that the T.I. 245 type of resistance to these had been incorporated also in the new line. The 6 additional viruses were those of cucumber mosaic, turnip mosaic, potato mottle, tomato ringspot, tobacco ringspot, and tobacco streak. This simultaneous inheritance of resistance to 7 viruses supports the hypothesis that a single genetic mechanism is responsible for all 7 resistances and that any 1 of the 7 will prove adequate in testing for the resistance applicable to the others.

STUDIES ON THE OVERWINTERING OF XANTHOMONAS VESICATORIA IN NEW JERSEY—G. D. Lewis and D. H. Brown—Pepper leaves infected by *Xanthomonas vesicatoria* were placed in nylon mesh bags in October. One bag was placed in each of the following locations: 1) the soil surface, 2) 1 in. below the soil surface, 3) 6 in. below the soil surface, 4) 12 in. below the soil surface, 5) under the roof of an open shed, 6) a greenhouse, 7) an unheated attic, and 8) the writer's office. In May the bags were collected. The contents of each bag were soaked in water. The resulting suspensions were used to inoculate young pepper plants. The plants were inoculated by placing the leaves in the bacterial suspension in a vacuum chamber. Air was withdrawn from the chamber until bubbles left the leaves. The plants were then removed from the chamber and placed in a greenhouse. Symptoms were apparent in 5 days. The suspensions from locations 7 and 8 produced abundant infections. The suspensions from locations 4, 5, and 6 produced scattered infections. No infections developed from the other suspensions. *X. vesicatoria* was isolated from within pepper seeds that were soaked in a suspension of the pathogen prior to surface sterilization of the seed.

WILT DISEASE OF GOLF GREENS AND ITS CONTROL WITH NABAM—R. J. Lukens and E. M. Stoddard—Leaves of bentgrass on Connecticut golf greens wilt during hot summer days despite adequate soil moisture. Golfing on these greens badly damages the wilted leaves. Wilted grass plants have roots that are only 1-2 in. deep and heavily infected with a *Rhizoctonia* sp. The condition was corrected by injecting the diseased turf to a depth of 1 ft with a 0.016% solution of commercial nabam formulation (22% disodium ethylenebis (dithiocarbamate) under a pressure of 200 lb/in.^{2} This treatment reduced *Rhizoctonia* infections and stimulated the growth of new roots. In addition to these effects, the pressure treatment also aided root growth by breaking up a compacted soil layer 2-4 in. below the surface of the green. Diseased turf treated in 1959 developed roots 12 in. deep within 2 months, and showed no wilt symptoms during the summer, 1960.

INTERNAL SPOTTING OF COCONUTS—Karl Maramorosch, Hans Rothkirch, and Bernardo S. Castillo—A previously unknown disorder of coconuts (*Cocos nucifera*) was found on the island of Mindoro, Philippines. A large percentage of nuts shipped from that island to San Pablo, Luzon, were affected by "internal spotting." Dark-gray and yellowish-gray spots were found on the outer side of the coconut meat after removal of the seed skin. The spots did not penetrate deeper than 2-3 mm and remained unnoticed in unpeeled nuts as split for copra. Peeling of the meat is a necessary part of the shredding process in modern desiccating factories, but is not required elsewhere. Spotted nuts are unsuitable for desiccation but can be used for copra, although the oil from such nuts is cloudy and of inferior quality. An inspection of plantations on Mindoro in the vicinity of Pinamalayan revealed that certain trees, with a high incidence of internal spotting in 1957, had mostly normal nuts in 1960. Nearby trees had up to 90% of spotted nuts. This suggests that recovery may occur. A study of nuts from other islands revealed the occasional occurrence of internal spotting in places far removed from Mindoro. The cause of this disease is unknown. The high incidence of spotting on Mindoro as opposed to other islands has not been explained.

EFFECT OF FUNGICIDES ON THE LARVAL EMERGENCE OF THE TOBACCO CYST NEMATODE—P. M. Miller, G. S. Taylor, and E. M. Stoddard—The following fungicides, added to infested soil in the laboratory, stimulated the emergence of larvae from cysts of the tobacco cyst nematode: 1) Diamond Alkali 649 (95% 3,3,4,4-tetrachlorotetrahydrothiophene-1,1-dioxide); 2) Puratized Agricultural Spray (5% tris (2-hydroxyethylphenylmercuri) ammonium lactate); 3) General Chemical 2466 (50% bis (3,4-dichloro-2-(5) furanonyl) ether); 4) Chemagro D-113 (20% 1,2-dichloro-1-methylsulfonyl ethylene); 5) Dithane D-14 (22% disodium ethylenebis (dithiocarbamate); 6) Dithane M-22 (80% manganous ethylenebis (dithiocarbamate); and 7) Amobam (42% ammonium ethylenebis (dithiocarbamate). As little as 6 ppm of Diamond Alkali 649 increased emergence 3 to 5 times in 21 days. 2, 4 or 5 at 2000 ppm increased emergence 3 to 5 times in 24 hours. More than 67 ppm of 1 or 200 ppm of 4 not only stimulated emergence but also killed the larvae after they emerged. Sulfur, at 2000 ppm, dodine (65% n-dodecylguanidine acetate), chloranil (50% tetrachloro-*p*-benzoquinone), 15% streptomycin sulfate, and filipin each retarded larval emergence in the laboratory. In the field, treatments 1, 3, 4, 5 and 6 increased emergence up to 10-fold within 3 weeks.

These fungicides may act directly on the eggs or indirectly by changing soil microflora.

USE OF POLYETHYLENE BAGS FOR CORN ROOT INFECTION STUDIES—R. E. Miller and C. W. Boothroyd—A modification of the method reported by H. den Ouden (1958) for observation of nematode activity around plant roots was used in a study of fungal infection of roots of corn seedlings. Polyethylene bags, sterilized in 1% NaClO, were filled with warm agar, which was pressed into a 3-mm layer. The top of each bag was fitted to a wooden collar plugged with cotton. Germinated corn seeds were transferred aseptically to the upper portion of the solidified agar. Inoculum was introduced into the agar in the vicinity of a root by means of a hypodermic syringe or by insertion through a cut section of the bag. Five genera of fungi (*Fusarium, Gibberella, Penicillium, Pythium,* and *Trichoderma*) isolated from corn roots were used in 2 experiments, one involving growth of seedlings in agar at pH 6 for 24 days at 22-24°C and the other in agar at pH 7 for 10 days at 10°C and then at 22-24°C for 14 days. Growth of uninoculated checks was less in the pH 6 series than in that at pH 7. *Fusarium, Gibberella,* and *Trichoderma* each produced more root rot (clearly visible through the polyethylene) under the conditions of the pH 6 series than under those of the pH 7 series. *Pythium* had little effect on roots in the tests at pH 6 but caused severe rot at pH 7. *Penicillium* induced no rot in any of the tests.

INFLUENCE OF NUTRITION, PH, SOIL TEMPERATURE, AND SOIL MOISTURE ON PYTHIUM BLIGHT OF HIGHLAND BENTGRASS—L. D. Moore, H. B. Couch, and J. R. Bloom—Highland bentgrass (*Agrostis tenuis* Sibth.) was grown in 8 in. glazed crocks with silica sand as the supporting medium. Using Hoagland's (H) solution, various nutrient and pH levels were supplied through a continuous-flow drip culture system. The plants were inoculated with aqueous mycelial suspensions of *Pythium ultimum* Trow. As compared with balanced nutrition (1.0 H), disease development was less at low nitrogen (0.1 *N*) and low balanced (0.1 H) nutrition levels. At 0.0, 0.1×, and 3.0× calcium, disease development was higher. Susceptibility increased when the plants were grown at 1.0 H and 3.0 H nutrition at pH 9.0. With 0.1 H at pH 9.0, however, disease development was less. Using 70°F as a standard, soil temperature of 50°, 60°, and 80°F did not alter the disease proneness of plants grown at 1.0 H at pH 5.6, 0 calcium at pH 5.6 and 1.0 H at pH 9.0. The influence on disease development of 3 soil moisture levels in the readily available range was studied by varying frequency of irrigation prior to inoculation. Disease development was greatest when the plants were allowed to extract to the permanent wilting percentage before irrigating back to field capacity.

LEAFROLL OF TOMATO CAUSED BY A STRAIN OF POTATO LEAFROLL VIRUS—J. J. Natti and W. T. Schroeder—In 1952, in a commercial planting of Rutgers tomato near Geneva, N. Y., 75% of the plants were infected with what appeared to be an unreported virus disease. The plants originated as transplants from North Carolina. Older leaflets of infected tomato plants were normal in size but rolled upward along the margins. Young leaflets curled downward and inward along the margins and particularly at the tip. A yellowing of the leaflet margins which appeared as a distinct narrow border was the most typical symptom. Terminal leaflets were reduced in size and gave the plants a spindly appearance. The disease caused a marked reduction in both size and number of fruit set, but did not affect the color or shape of the fruit. Transmission, host range, and cross-protection tests indicated the causal virus to be a strain of potato leafroll virus. Its severe reaction on tomato and *Nicotiana rustica* L. distinguished it from other potato leafroll viruses.

THE PHYSIOLOGY OF 'LYCOMARASMIN' PRODUCTION BY FUSARIUM OXYSPORUM F. CUBENSE—O. T. Page—A peptide-like toxin (N-(α(α-hydroxypropionic))-glycylasparagine) was named 'lycomaramin' after initial isolation from the tomato-wilt fungus (*Fusarium lycopersici*). At the outset of the present investigation, it was postulated that 'lycomarasmin' was a product of the macro-species, *F. oxysporum.* This expectancy has been realized by the identification of this peptide- substance in the banana-wilt fungus (*F. oxysporum* f. *cubense*). 'Lycomarasmin', together with its decomposition product "Substance I', was present in filtrates of 5-day-old cultures as well as in 100-day-old culture filtrates grown on modified Richard's medium. 'Lycomarasmin' and its decomposition products were identified by means of a Spinco/Beckman Amino Acid Analyzer. The persistence of the peptide in culture filtrates was in sharp contrast to the disappearance of amino acids shortly after their liberation with culture solution. Present evidence indicates that 'lycomarasmin' is a constituent of the cell walls of *F. oxysporum.* Liberation of the peptide was achieved by mild alkaline hydrolysis of ether extracted cell-wall preparations.

COMPARATIVE PATHOGENICITY STUDIES OF FUNGI ASSOCIATED WITH CORN STALK ROT—J. L. Peterson—In 1959 several fungi were isolated from stalk-rotted corn approximately 5 weeks after the corn was at full silk. These organisms included, in addition to *Gibberella zeae,* species of *Helminthosporium, Trichoderma, Penicillium, Curvularia, Mucor,* and *Fusarium.* In 1960 2 inbred corn lines, one apparently a line of good standability (resistant) and the other a line of poor standability (susceptible), were inoculated with 17 selected isolates of these organisms. Inoculations were made by inserting infested toothpicks into the second expanded internode when the corn was at full silk. Studies conducted under these conditions indicated that organisms not commonly thought to be associated with the stalk rot were equally or more pathogenic than many isolates of *G. zeae.* There was little difference in the extent of rot caused by the various fungi tested in the resistant inbred. Rot was much more extensive in the susceptible inbred with all organisms. The *Trichoderma* sp. which were readily isolated from corn in 1959 resulted in a more extensive rot than other organisms tested in 1960. *Helminthosporium* sp., *Curvularia* sp., *Fusarium moniliforme,* and other *Fusarium* sp. caused approximately the same amount of rot as strains of *G. zeae.*

CONTROL OF DISEASE SYSTEMICALLY BY ROOT ABSORPTION OF DERIVATIVES OF 2-PYRIDINETHIOL-1-OXIDE—Samuel S. Ristich and Sylvan I. Cohen—While many derivatives of this compound are leaf absorbed and protect untreated leaves systemically, relatively few are effective by root absorption. Certain alkyl oxygen- and thio-ether derivatives are most effective, producing 85-99% control of cucumber anthracnose and about 80% control of tomato early blight after a single 50 ppm soil drench. Chemical 2235 (1-oxido-2-pyridylthiomethyl propyl ether), assigned the common name of propoxymethyl pyridinethione, retains its systemic activity in cucumber at least 7 days after soil application. It exhibits superior translocatability by protecting both leaves of a cucumber plant when griseofulvin at equal concentration only protects the oldest leaf. However, at 50-100 ppm, 2235 causes stunting and marginal leaf necrosis of these 2 plant species. Evaluation of 10 analogs shows that the short carbon chain ethers are highly systemic but also phytotoxic. A single thio-ether derivative tested showed good activity and no injury. All derivatives were superior to the parent compound which is highly unstable. Effectiveness by soil drenching was not correlated with water solubility.

VECTOR SPECIFICITY OF THREE STRAINS OF BARLEY YELLOW DWARF VIRUS—W. F. Rochow—Three naturally occurring vector-specific strains of barley yellow dwarf virus were differentiated in comparative trans-

mission tests with 4 virus isolates that induce similar symptoms in oats and with *Macrosiphum granarium* (MG), *Rhopalosiphum padi* (RP) (previously called *R. fitchii*), and *R. maidis* (RM). Aphids were given an acquisition feeding of 1-2 days, each species on a separated portion of the same detached leaf, and then transferred in groups of about 10 to oat seedlings for a 3-day inoculation test feeding. In 10 parallel serial transfers made over a period of 1 year, the first isolate was transmitted to 69 of 72 plants by MG, to 0 of 60 by RP, and to 0 of 60 by RM; the second isolate to 0 of 60 by MG, to 62 of 69 by RP, and to 0 of 60 by RM; and the third isolate to 0 of 60 by MG, to 0 of 60 by RP, and to 26 of 66 by RM. The fourth isolate, differing from the third in that it was obtained from barley instead of oats, was transmitted to 0 of 45 plants by MG, to 0 of 45 by RP, and to 28 of 51 by RM. Specificity was not absolute when acquisition feeding was for 4-5 days on intact plants; each virus strain was transmitted not only by its regular vector but also occasionally by 1 or both of the other aphid species.

TOXICITY OF PLANT SAPS TO THE NEMATODE, PANAGRELLUS REDIVIVUS—G. J. Stessel and P. E. Sakkinen—Expressed sap from 17 of 54 plant species tested was toxic to the nematode, *Panagrellus redivivus* (Linn, 1767) Goodey, 1945. The plant parts were crushed at 12,000 lb/in^2 and the toxicity of the crude sap was determined after 4-, 24-, and 48-hour exposures of worms. The nematoxicity of expressed juices from the following plants is indicated by the order of listing from most potent to least: *Tagetes lucida* Cav. (roots), *Phytolacca decandra* L. (roots), *Arctium minus* Bernh. (roots), *Calendula officinalis* L. (roots), *Tropaeolum majus* L. (aerial), *Erucastrum gallicum* (Willd.) O. E. Shulz (whole plant), *Ambrosia trifida* L. (roots), *Polygonum cuspidatum* Sieb. & Zucc. (roots), *Solanum dulcamara* L. (berries), *Rhus typhina* L. (aerial), *Scirpus cyperinus* (L.) Kunth. (aerial), *Saponaria officinalis* L. (aerial), *Ambrosia artemisiifolia* L. (aerial), *Verbena hastata* L. (aerial), *S. dulcamara* L. (aerial), *Lycopodium complanatum* var. *flabelliforme* Fernald (whole plant), *Fagopyrum esculentum* Moench. (roots), and *Portulaca oleracea* L. (roots).

THE PROBLEM OF GERMINATING UREDOSPORES—Riksh Syamananda and Richard C. Staples—The difficulty in obtaining an adequate quantity of germinated uredospores of rust fungi has long been realized by uredinologists. A satisfactory method of germinating uredospores of bean and corn rust fungi in bulk has been devised. Two hundred mg of freshly collected dry uredospores were dispersed over a water surface for 2 hours and the liquid removed by filtration. Prefloated spores were resuspended in a Fernbach flask containing 0.01% Tween 20 and the flask was shaken vigorously on a reciprocating shaker. All operations were executed in light at 20°C. Over 98% germination was obtained under the prescribed conditions. Factors critical for good spore germination were storage period, prefloating time, temperature, spore titre, self-inhibitor, and natural stimulator.

TOBACCO FLECK CONTROLLED WITH ANTIOZONANTS—G. S. Taylor and S. Rich—Fleck caused more than $1,000,000 damage to the Connecticut tobacco crop in 1959. Fleck appears after hot, hazy days when atmospheric ozone exceeds 0.05 ppm. In 1960, field plots of Connecticut 49 tobacco were sprayed or dusted with NBC (nickel-di-N-butyldithiocarbamate) either weekly or on days of predicted high ozone. When applied on a prediction basis, NBC gave 95% control of fleck at 6.0 lb and 72% control at 1.3 lb of compound per acre. Applied weekly, NBC gave 86% and 56% control at 6.0 and 1.3 lb of compound per acre respectively. Technical NBC was not phytotoxic. As a wettable powder (65%), NBC was phytotoxic in sprays more concentrated than 0.25%, or in dusts containing more than 30% of the formulation. The effect of NBC on tobacco leaf quality is now being examined. A 0.2% spray of Santoquin (6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline) reduced fleck 48% when applied on high ozone days, but was ineffective if applied more than 1 day before the fleck outbreak. Shade cloth tents treated with Tenamine 30 (N,N'-bis(1-methylheptyl)-*p*-phenylenediamine) reduced fleck on enclosed plants by 70%. The treated cloth remained effective about 10 days. These results provide additional evidence that ozone is involved in tobacco fleck.

THE INHIBITORY ACTIVITY OF COCONUT MEAT EXTRACT AND THE JUICE OF AGROPYRON REPENS ON THE INFECTIVITY OF TOBACCO MOSAIC VIRUS—V. Weeraratne and A. E. Rich—Coconut meat extract reduced the infectivity of tobacco mosaic virus (TMV) by 92%, when assayed on *Nicotiana glutinosa*, employing the half-leaf method, whereas coconut milk reduced the infectivity by only 39%. The juice of *Agropyron repens* leaves and stems reduced the infectivity of TMV by 93%. The storage of coconut meat extract and juice of *A. repens* for 32 days at room temperature and at 4°C had no effect on their inhibitory action. Application of coconut meat extract 8 days prior to the inoculation of the leaves with the virus had the same inhibitory effect as the fresh extract. Coconut meat extract applied to the leaves 12 hours after the inoculation with the virus resulted in only 32% inhibition of infection, and *A. repens* juice applied in the same manner inhibited infection by only 12%. Ten-fold dilution and 10-fold concentration of coconut meat extract had no effect on inhibitory action, whereas 100-fold dilution reduced the inhibitory activity by 32%. Thousand-fold dilution completely removed the inhibitory action, whereas the 10,000-fold dilution increased infectivity by 14%.

PHYTOPATHOLOGICAL NOTES

THE POSSIBLE RELATION OF MANGANESE TO STEM CANKERS IN RED OAK

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Stem cankers on northern red oak (*Quercus rubra* L.) and other forest trees were associated by True and Tryon (6) with the summer drought of 1953. In a sampling survey of 33 West Virginia counties during the summer of 1957, similar cankers on oak were widespread.

A significantly higher average concentration of manganese was found in the bark that covered these cankers (1239 μg/g) than in apparently healthy bark of cankered trees (977 μg/g). This in turn exceeded the average manganese content of the bark of noncankered trees growing at sites with some diseased trees (789 μg/g). The bark of healthy oaks at sites free of cankered trees contained the least manganese (455 μg/g). These average concentrations are indicated graphically in Fig. 1. The calculated F value (22.27) provides evidence that the differences are significant at the 1% level. Leaves, stems, and roots contained progressively lower concentrations of manganese, as other investigators have shown (3, 4); and the manganese content of bark tissue always exceeded that of

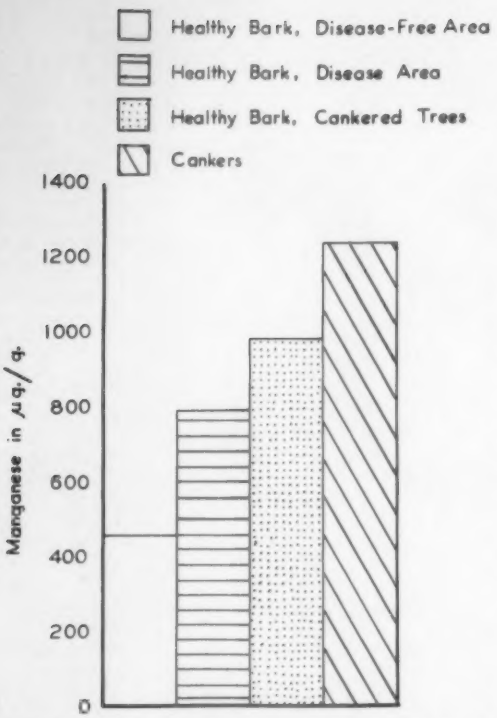

Fig. 1. A comparison of manganese concentrations in diseased and nondiseased trees. These differences were significant at the 1% level.

adjacent wood. Chemical analyses for manganese were carried out by a modification of the method of Willard and Greathouse (7).

ATTEMPTS TO INFLUENCE MANGANESE CONCENTRATION OF STEM BARK.—An attempt was made to determine the effect of lethal mechanical injury upon the manganese concentration of the tissues. Pounding the bark with a blunt instrument caused no appreciable change in manganese content during the 104-day period of intermittent sampling and analysis that followed wounding.

The introduction of manganous sulfate into cambium-depth wounds as a powder (1.5 g/wound) or into the xylem as a solution (100 ml of concentrations up to 1,500 ppm) produced no cankers.

In another attempt to induce canker formation, 80 red oaks, growing in the forest at 3 different sites, were treated with sulfur and with calcium hydroxide to induce differences in soil pH. Different quantities of manganous sulfate were applied to the soil.

One site, designated Streamside, was situated close to and somewhat above a small stream where the average available soil moisture (with permanent wilting point taken as 0 and field capacity as 100) was 87.2% at the 4-in. depth, as measured from Bouyoucos soil moisture blocks (2) by the method of Rowland, Fagan, and Crabb (5). Another site, designated Slope, on a moderate slope 0.25 mile from any stream, had an average available soil-moisture of 56.5% The third site, designated Dry Ridge, had its natural dryness accentuated by having a trench 18 in. deep dug around each tree 10-15 ft. from it. Waterproof roofing paper covered the area around each tree bounded by the trench. The average available moisture for the Dry Ridge plot was 47.9%. Moisture determinations were based on readings made from Bouyoucos soil-moisture blocks between June 15 and October 1, 1957. Since the purpose was to determine whether differences in soil moisture did exist among the 3 sites and because the method of Rowland, Fagan, and Crabb does not require it, the soil blocks were not calibrated. Calibration also was nonessential in this case because of the similarity among the soils, all of which were silt loams (stony phase). Dry Ridge and Slope sites were residual soils of the Dekalb series. Streamside was a colluvial soil of the Ernest series. The results indicate that considerable differences in available soil moisture existed among the 3 plots during the growing season. When bark samples taken from trees on the 3 sites were analyzed for manganese, the differences associated with site were small and not statistically significant.

Soils treated with sulfur on the 3 sites attained pH's of 3.8-4.3. Nontreated soils had a pH of 4.4 and 2 levels of treatment with calcium hydroxide induced soil pH's, respectively, of 4.5-5.5 and 5.5-6.5. When bark samples taken from trees growing at these different pH ranges were analyzed for manganese, the average concentrations of manganese were as follows: pH 3.8-4.3, 880 μg/g; pH 4.4 (unadjusted soil), 595 μg/g; pH 4.5-5.5, 573 μg/g; and pH 5.5-6.5, 563 μg/g. Only the manganese concentration at the lowest soil pH differed significantly from the others at the 1% level. This induced high manganese concentration (880 μg/g) was well above the average concentration of manganese in the bark of healthy nontreated trees growing on natural disease sites (789 μg/g), but it was not as great as the average manganese concentration found in the noncankered bark of cankered trees (977 μg/g).

The concentrations of manganese in the bark of trees growing on soils to which manganese had been added at the rates of 1, 3, and 5 lb of manganese/A (added as manganous sulfate) did not differ statistically from the 659 μg/g average manganese concentration of the bark of trees growing on soils to which no manganese had been added. No cankers developed on any of the experimental trees.

Although the relationship of manganese to drought-associated stem cankers in red oak is reminiscent of the findings of Berg, Clulo, and Orton (1) in studies of internal bark necrosis of apple, the cause of these stem cankers on oak remains undetermined. The presence of high manganese concentrations in canker-killed bark and in the healthy (noncankered) bark of cankered trees, however, has been established.

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A COMPARISON OF WOUND-TUMOR AND TOBACCO CLUB-ROOT

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This research was supported in part by a grant from the American Cancer Society to Dr. L. M. Black.

Tobacco club-root was first reported by Valleau and Johnson (8) in 1932. In 1947, Valleau (7) noted that diseased plants showed distortion of the leaves and tumors on the roots, and described tobacco club-root as a "wound-tumor-like disease." Black [see Valleau (7)] also noted the similarity between tobacco club-root and wound-tumor in tobacco and stated that they might be caused by related viruses. Some years later, Smith (5), in his book on plant virus diseases, listed tobacco club-root as caused by a strain of wound-tumor virus (WTV). It has been classified as a strain or suspected strain of the same virus in the list of common names of virus diseases compiled by the Commonwealth Mycological Institute (6). This study compares tobacco club-root and wound-tumor in similar hosts to determine whether tobacco club-root virus (TCRV) was indeed as closely related to WTV as had been suggested previously.

Tobacco club-root in *Nicotiana tabacum* L. was kindly supplied by Dr. Valleau, along with seed of a number of tobacco varieties. Wound-tumor was obtained in solanaceous plants by inoculation into tomato (*Lycopersicon esculentum* Mill. var. Bonnie Best) with viruliferous *Agallia constricta* Van Duzee (previously fed on diseased crimson clover, *Trifolium incarnatum* L.) and transmitted to *Nicotiana* spp. by grafting. The diseased *T. incarnatum* material was kindly provided by Dr. L. M. Black. Both wound-tumor and tobacco club-root were studied in *N. tabacum*, *N. glutinosa* L., and *N. rustica* L.

Symptoms of Tobacco Club-Root.—In *N. tabacum* varieties, severe top symptoms were seen 2 weeks to 3 months after grafting with tobacco club-root scions. Marked stunting of the main shoot, shortening of internodes, leaf curling, and leaf distortion were typical symptoms on *N. tabacum* and the other *Nicotiana* spp. In *N. tabacum*, the acute stage of the disease (Fig. 1) usually was followed by a much milder chronic stage (Fig. 2). Occasionally, a relapse to the acute stage occurred in subsequent growth. In many cases, vein enlargement and enations were observed on the underside of the leaves, but these were not the first noticeable symptoms. Root tumors of varying size and number were observed, but they were not directly related to severity of top symptoms. Great variation in tumor response was noted especially in *N. tabacum* varieties. Of the 3 *Nicotiana* spp. tested, *N. tabacum* showed the largest tumors.

In *N. glutinosa*, tobacco club-root symptoms were often seen as early as 11 days after grafting. Marked epinasty, stunting, and leaf necrosis occurred. Most plants died about 2 weeks after the first signs of infection. Root tumors, when present, were small.

In *N. rustica*, symptom expression was noted only after relatively long periods of time. In some cases, severe stunting, curling, and distortion of the leaves were seen 3 months after the initial inoculation (Fig. 3).

Symptoms of Wound-Tumor.—In contrast to tobacco club-root, the principal and most common shoot symptom of wound-tumor, in *N. tabacum* and the other *Nicotiana* spp., was the presence of vein enlargement and enations on the underside of the leaves. Stunting or distortion of the leaves and shoot was usually mild or did not occur. The development of a chronic stage in infected plants was not uncommon. Shoot symptoms were first noted after 2-3 months on *N. tabacum* and *N. rustica*.

On *N. glutinosa*, symptoms often were not observed until 4 or more months after inoculation. In *N. glutinosa*, WTV caused an increased production of axillary shoots which showed distortion and leaf enations. Tumor formation at the graft union was observed in 3 plants.

In *N. rustica*, shoot symptoms consisted of minor vein enlargement and leaf enations, with little or no distortion or stunting (in marked contrast to the symptoms of tobacco club-root on the same host).

Other Studies.—In the case of both wound-tumor and tobacco club-root, not all hosts which were successfully grafted developed symptoms. Grafted *N. tabacum* and *N. glutinosa* plants showed higher rates of symptom response (TCR-70%, WT-50%) than did grafted *N. rustica* plants (TCR-55%, WT-25%). Variations in plants, all of which were grown from seed, or variable virus content of scion material might explain such results.

Results of 2 experiments have indicated that *A. constricta*, a vector in which WTV multiplies (1), did not transmit WTV or TCRV from tobacco to crimson clover (*Trifolium incarnatum* L.). In the same experi-

ments, WTV was transmitted from crimson clover to crimson clover. Therefore, the relative ability of *A. constricta* to transmit TCRV or WTV was not determined.

Fig. 1-3. 1) Acute symptoms of club-root in *Nicotiana tabacum* var. Kentucky 153. The plant was inoculated by graftage on September 3, and photographed October 18, 1957. (×0.25.) **2)** Chronic symptoms of club-root in the uppermost leaves of *Nicotiana tabacum* var. Dixie Bright 101. This plant also was inoculated on September 3 and photographed October 18, 1957. Acute symptoms still show in the severely curled leaves some distance from the tip. (×0.2.) **3)** Acute symptoms of club-root in the apex and axillary branches of a *Nicotiana rustica* plant. (×0.2) Symptoms of wound-tumor on *N. rustica* are mild and difficult to distinguish from normal structures in a photograph.

Serological tests with the 2 viruses were attempted. Since tobacco club-root was not obtained in sweet clover, the tests were carried out with infected *N. tabacum*. Root tumor, mid-vein, and petiole tissue of *N. tabacum*, infected with wound-tumor or tobacco club-root, provided soluble antigen preparations or their equivalent, by the density-gradient techniques of Brakke (2, 3, 4). These were tested against antiserum to wound-tumor virus by the ring test. Only the root-tumor tissue from wound-tumor tobacco reacted positively and even in this case only to a dilution of 1:16 in terms of the starting weight of tissue. Although not conclusive, the tests provided some evidence for lack of serological relationship between the 2 viruses.

In view of the differences in symptom expression, particularly in *N. rustica*, and the failure to detect a serological relationship, it is concluded that TCRV is probably not a strain of WTV, but is an unrelated virus whose symptoms in some *Nicotiana* spp. are somewhat similar to those of wound-tumor.

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DEPOSIT RATE AND SPRAY OIL COMPOSITION IN RELATION TO PHYTOTOXICITY AND SIGATOKA DISEASE CONTROL ON BANANA LEAVES

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In previous studies (1, 2) on oil sprays for the control of the Sigatoka leaf-spot disease of bananas (*Mycosphaerella musicola* Leach), the naphthenic oils tested were superior to similar paraffinic oils. These results were based on trials conducted at the narrow deposit range of 2.1-2.8 mg of oil/100 cm^2 of leaf surface, which was the median deposit obtained in an earlier test (3) with a motorized knapsack mistblower applying 1.5-2.0 gal/A. Appreciable amounts of oil are deposited, however, above and below the median value. The purpose of this study was to determine whether a naphthenic oil is superior to a paraffinic oil at such deposit levels.

A naphthenic and a paraffinic spray oil were employed, having characteristics (2) similar to commercial oils. They were alike in unsulfonated residue (94-95%) and viscosity (74-75 SUS/100°F). Four applications at 2-week intervals were made, using a precision spray technique (3), on selected leaf-halves of Dwarf Cavendish plants, during a period of severe disease. Only the upper leaf surface was sprayed, because other trials showed that with knapsack or aerial sprayers the lower leaf surface usually received negligible amounts of oil.

Data were taken 2 weeks after the final spray. Leaves were observed for phytotoxic flecking symptoms and for disease incidence. Phytotoxicity was rated as the percentage of sprayed leaf area covered with flecks. Disease incidence was rated on both sprayed and unsprayed leaf-halves. For each leaf, the percentage of the sprayed area with disease was divided by the percentage of the unsprayed area with disease. A disease rating of about 1.00 indicates no control; whereas a low disease rating, especially under 0.10, indicates good disease control by the oil treatment.

A trial was conducted to determine the relationship of 5 deposit levels of the 2 oil types to disease control and phytotoxicity. The deposit levels used were 1, 2, 3, 4, and 5 mg of oil/100 cm^2 of leaf surface, which include amounts greater and less than those tested previously (1, 2). The first spray was applied to the most recently unfurled leaf on each of 100 plants. Fifty leaves were used for each oil, 10 replicates/deposit. The treatments were arranged in a randomized block design.

The phytotoxicity and disease incidence results are presented in Fig. 1. Each point represents the average value for 10 replicate leaves. Phytotoxicity (percentage of flecking) caused by the naphthenic oil was 0 at the 1-mg deposit level; it increased gradually to 28% at 5 mg. The amount of phytotoxicity caused by the paraffinic oil was higher at all deposits; it was 3% at 1 mg, increased rapidly to 24% at 2 mg, and continued upward gradually to 43% at 5 mg. Analysis of variance showed a highly significant difference between the phytotoxicity results of the 2 oils at each deposit level tested.

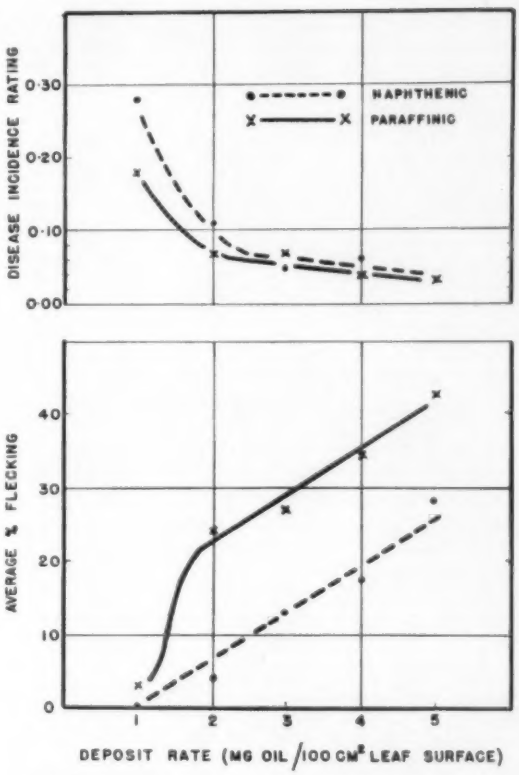

Fig. 1. Sigatoka disease incidence (top) and phytotoxic flecking (bottom) on banana leaves sprayed with comparable naphthenic and paraffinic oils at different deposit rates.

The disease incidence value for the naphthenic oil was 0.28 at the 1 mg deposit level; it fell rapidly to 0.11 at 2 mg and decreased gradually to about 0.05 or less at the higher deposits. The disease incidence value for the paraffinic oil was less at the first 2 deposits—0.18 at 1 mg and 0.07 at 2 mg—it then tended to decrease gradually at the higher deposits. Analysis of variance, however, showed no significant difference between the disease incidence results of the 2 oils at any of the deposit levels tested.

The disease incidence curves for the 2 oils reached a low value (indicating good disease control) at the deposit range of 2-3 mg of oil/100 cm^2. Further increase in oil deposit did not give appreciably better disease control. If the 2 phytotoxicity curves are compared at 2 and 3 mg, it can be seen that the naphthenic oil caused only 20% and 50%, respectively, as much phytotoxic flecking as the paraffinic oil.

These results show that the naphthenic oil is superi-

or to the paraffinic over a broader deposit range than that tested previously (1, 2). In this broader range of 1-5 mg, the naphthenic oil is significantly less phytotoxic than the paraffinic, whereas disease control is essentially the same for both oils at the 3-5 mg levels. At lower deposits, the disease curves diverge. Although the differences between the 2 oils at 1 and 2 mg are not statistically significant, the divergent trend may be real, indicating that the naphthenic oil will give less disease control than the paraffinic at very light applications. With the possible exception of very light deposits, therefore, the naphthenic oil tested here is preferable to the paraffinic for low-volume spraying of bananas.

The lowest oil deposit necessary for good Sigatoka control was not known previously. For both of the oils tested, the minimal amount is approximately 2-3 mg/100 cm^2 when applied at 2-week intervals during a period of high disease incidence. It is possible that differences in oil quality, disease potential, or spray intervals may change the minimal oil deposit required for good disease control. For 1 set of field practices and perhaps for others, however, these results do establish a minimal deposit level for Sigatoka control.

Investigations on oil characteristics and deposit rate for spraying bananas have been rewarding. The data from this and earlier experiments (1, 2) demonstrate that good Sigatoka disease control with a minimum of phytotoxicity can be achieved with a naphthenic oil having a viscosity of 75-85 SUS (at 100°F) and employing a deposit rate of 2-3 mg/100 cm^2 of leaf tissue. These results show that small-scale trials with precise techniques can be useful in determining which oils and application methods are best for low-volume spraying of bananas.

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INJURIES ON CANTALOUPE LEAVES ASSOCIATED WITH LAMINAL GUTTATION AWAY FROM MARGINAL HYDATHODES

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In 1804, the Swiss plant physiologist, De Saussure (7), described a plant disease caused by the action of salts and other products formed on the periphery of leaves as a result of guttation. The "Maladie Blanche" which he observed on many garden plants constitutes a kind of nonparasitic disease overlooked until much later (1, 2, 3, 4). The injuries described thus far, however, have been chiefly on leaf margins of the tipburn type, beginning at the location of the hydathodes. Less known are other exudation phenomena that take place through the epidermal system of the leaf lamina away from the hydathodes, which also may be associated with injury (5, 9).

In general, laminal guttation is much less conspicuous but seems much more frequent than guttation through marginal hydathodes. It has been observed by this writer on many species of plants at different times and locations. The guttating surface area may vary from small, almost microscopic, to large, sometimes comprising several cm^2, or even an entire leaf blade. It becomes covered with a thin, glistening film of a slightly sticky substance, apparently formed by the coalescence of many small drops. After the liquid evaporates, various crystalline or amorphous lumps may be seen by the naked eye or microscopically (Fig. 1-A). These bodies may take up atmospheric moisture and become reabsorbed by the plant, thus causing injury on susceptible tissue; or they may become insoluble and remain on the surface indefinitely. Such deposits have been seen in conspicuous quantities on citrus leaves and fruits, crape myrtle, and cantaloupes. In less conspicuous amounts, but more often, they have been observed on various legumes and grasses, including small grains and corn, in Texas, Arizona, New Mexico, Mississippi, Illinois, and Wisconsin. All these deposits were observed almost invariably on the exposed surfaces. Dews and rains seem to wash away the guttation drops or the freshly formed deposits. In this manner, some nutrient material may be lost by the plants.

The cantaloupe plant seems particularly sensitive to the presence of exudation products. Local necrotic spots followed by yellowing and blighting of entire leaves and petioles (Fig. 1-B) were observed for several seasons on vines grown on greenhouse benches. The spots originated at 1 or several of the guttation *loci* which may develop anywhere on the blade. The exudation usually took place in the early part of the morning. Sometimes, it was seen as a few small drops arranged in a circle several mm in diameter, with another drop in the center, as seen on pepper leaves in the field. Most often, however, the exudation was observed as a liquid film occupying several mm^2. In the afternoon, this liquid may have become slightly sticky. Some lumps of bluish-white deposits then were formed. Microscopic examinations always showed the leaf hairs coated with the solidified exudate, suggesting that guttation may have taken place through the numerous pits found on these organs. These developments may have been repeated for several mornings at the same *locus*. Two or more days later, a water-soaked area appeared which in time turned necrotic. Some-

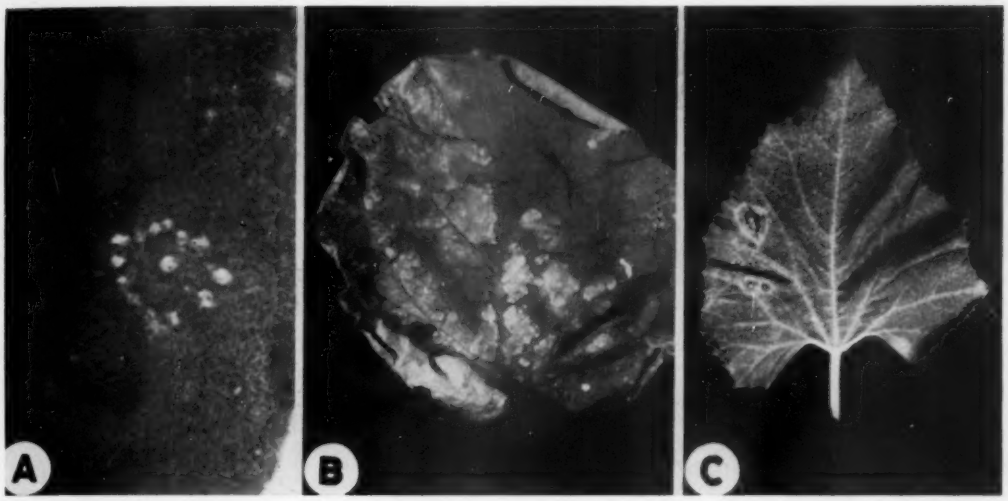

Fig. 1. Chemical deposits and injuries following guttation. **A)** Pattern of arrangement of small lumps of guttation deposits following evaporation of guttation drops on a leaf of pepper as found on cantaloupe leaves prior to development of injury. **B)** Extensive filmy deposits on a cantaloupe leaf. **C)** Beginning of guttation leaf blight in cantaloupes. Note local necrotic lesions surrounded by rings. In some varieties the rings are brown. In time, such leaves turn yellow and die. (**A** and **B** from field specimens; **C** from the greenhouse.)

times, this necrotic area was surrounded by 2 or more concentric rings (Fig. 1-C). In about a week or more, the entire leaf began to turn golden yellow; the discoloration spread from the lesion or lesions outward in all directions. Not all the leaves were so affected, but only the alternating ones on a particular runner. While the older leaves were in the process of being killed, the younger leaves became gradually and similarly affected. An entire plant is rarely killed, but its development and fruiting may be greatly delayed. Similar lesions were induced artificially by drop application of 4% Na_2HPO_4 solution and some other phosphorus salts.

The laminal guttation injury described here and the marginal guttation injury previously reported suggest the symptoms of "Crown Blight" of cantaloupes encountered in Arizona and California (6, 8). "Crown Blight" has been of considerable importance in these 2 states and its cause has not yet been definitely determined. Thus, it may be interesting to investigate its being partly of guttation origin, since guttation injuries seem a hazard developing primarily in arid regions of this country, devoted to winter agriculture. Since these troubles are not generally known, they may be overlooked or misinterpreted.

Both kinds of injuries, marginal and laminal, were experimentally induced under partially controlled conditions for guttation in the greenhouse on cantaloupe plants grown in poor soil as well as in soils containing potassium and phosphorus in excess. The plants grown on the richer soil suffered greater injury than those grown on the unfertilized soil. Possibly, however, the primary cause of guttation blight may be the unbalanced and excessive absorption of soil nutrients by the plant. In irrigated regions, excessive salts are frequently found and conditions for guttation are favorable.

It is not certain whether the forces involved in peripheral guttation through hydathodes are the same as those inducing laminal guttation at various local points on the leaf blade.

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NEWS

Editor: K. W. KREITLOW, Plant Industry Station, Beltsville, Md.

Congratulations to phytopathologist James H. Jensen who will asume the presidency of Oregon State University on Sept. 1. Dr. Jensen was Head of The Department of Plant Pathology, North Carolina State College from 1945 to 1953. Since 1953 he has been Provost at the Iowa State University.

The 1961 annual meetings to be held at Biloxi, Mississippi will be headquartered in the Buena Vista Hotel. According to Local Arrangements committee Chairman W. W. Hare, ample accommodations are available along the "Riviera of the South." Since this is a hotel meeting, there are no special facilities for wives and children and arrangements will have to be made individually. There will be a program for wives including commercial tours in the area. A "Southern Division Coffee Hour" is being planned around several of our talented phytopathologists. The banquet will be the Buena Vista's famed "Shrimp Jamboree" with other dishes available for those not wanting shrimp. Entertainment will be by the Hinds Junior College "Hi Steppers" of television renown.

Distinguished Professor of Plant Pathology, W. D. Valleau, retired July 1, 1961, after 42 years of service at the University of Kentucky, Lexington. Dr. Valleau is continuing work with diseases of tobacco and other crops.

Forest Pathologist Ellis B. Cowling of Yale University is spending the summer at North Carolina State College where he is investigating, in cooperation with Dr. Arthur Kelman, the nature of cellulolytic enzyme systems of certain phytopathogenic microorganisms and their role in tissue disintegration.

Congratulations to M. F. Kernkamp who was recently appointed Head of the Department of Plant Pathology, University of Minnesota. Dr. Kernkamp has served for several years as Assistant Director of the Agricultural Experiment Station. He succeeds J. J. Christensen who retired June 30.

Nematologist A. L. Taylor, Beltsville, Maryland was a United States representative at the 6th International Nematology Congress held at Ghent, Belgium, July 24 to 28. In conjunction with the Congress, Mr. Taylor will visit nematology laboratories in western Europe and England.

Phytopathologist Harvey W. Rankin retired July 1 as Head of the Department of Plant Pathology, University of Georgia Coastal Plain Experiment Station, Tifton, Georgia. He worked primarily with diseases of vegetable crops in the South Georgia area.

Citations for outstanding service were received by two members of the Department of Plant Pathology, University of Arkansas. In their first annual awards, the Arkansas alumni association bestowed acclaim and cash on Dr. D. A. Slack for distinguished professional service. Dr. Slack was one of three faculty members to receive an award for excellence in research and teaching. Dr. J. L. Dale was selected the outstanding teacher in the College of Agriculture and presented an appropriate award by the Alpha Zeta fraternity.

Plant Pathologist John M. Kainski began work July 1 in the Department of Botany and Plant Pathology, Kansas State University, as Assistant Professor of Botany. Dr. Kainski received his Ph.D. from Cornell in 1959 and will work on vegetable and fruit diseases, replacing Dr. O. H. Elmer who recently retired.

Nematologist O. J. Dickerson, a June 1961 graduate of the University of Wisconsin, began work July 1, 1961, as assistant professor of Botany at Kansas State University. Dr. Dickerson will do research on plant parasitic nematodes and will teach courses in plant nematology.

The XVIth International Horticultural Congress will be held in Brussels (Belgium) August 31st to September 8th, 1962. It is interesting to note that the First International Horticulture Congress was also held in Brussels in April, 1864. So, with a mere 20 months ahead of the actual date, it will thus be possible to celebrate the Centenary of the International Horticultural Congresses in the city where it originated.

Nematologist John P. Hollis, Department of Plant Pathology, Louisiana State University, has been awarded a Fulbright grant as a research scholar. Dr. Hollis will conduct soil fumigation experiments to determine the economic importance of plant parasitic nematodes in British East Africa. He will be stationed at the Scott Agricultural Laboratories in Kenya for a 9-month period beginning in September. Dr. Hollis will work with A. G. Whitehead of the East African Agriculture and Forestry Research Organization.

The North Central Division held its annual meeting June 26 and 27 on the campus of the South Dakota State College, Brookings. Tours of research installations and field plots were made. Registration for the meeting totaled 103 and 125 attended the annual banquet. Dr. H. M. Briggs, President of the College, presented the principal address on "A century of progress in the Land Grant Colleges." Officers elected were: President, Axel Anderson; Vice President, Richard Kiesling; Councilor, M. B. Linn; Secretary-Treasurer, Jack Wallin. The 1962 meeting will be held in June at Michigan State University, East Lansing.

Plant Pathologist Robert F. Bozarth, graduate student at Cornell University, has been awarded the first postdoctoral Research Associateship in the Agricultural Research Service Plant Virology Laboratory, Beltsville, Maryland. He will study amino acid metabolism of virus-infected plants.

The American Type Culture Collection has received a grant of $865,000 from the National Science Foundation for construction of a building to house the rapidly expanding facilities of the Collection. This sum, with $90,278 from the National Institutes of Health and $150,000 from private sources, makes a building fund of $1,105,278. Two acres of land have been purchased near Rockville, Maryland. Plans for the building, to provide 30,000 square feet of working space for about 50 staff members, are well under way. A greenhouse for plant virus work is to be included and ample parking and other facilities will be available. A second grant for $99,750 from the National Science Foundation provides for logistic support for the Collection for a 3-year period. A number of research projects involving taxonomic studies of selected groups of bacteria are already under way. The National Science Foundation has recently provided $61,000 for the study of fungus preservation techniques.

Phytopathologist Mohammed Myser Ali has joined the staff of Jamia College, Karachi, Pakistan, as Head of the Department of Botany. Dr. Ali recently received his Ph.D. degree in plant pathology at the Ohio State University, Columbus, Ohio.

Deaths—Charter member H. T. Güssow died June 15 in Victoria, B. C. at the age of 82. Dr. Güssow, who retired in 1944, was formerly Chief of the Division of Botany and Plant Pathology, Canada Department of Agriculture. Dr. Güssow served as President of the Society in 1935 and was one of two Canadians who have held this office.